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(54) Title: HUMANIZED CDR-GRAFTED ANTI-ICAM-1 ANTIBODIES, METHODS OF PREPARATION AND USAGE THEREOF

(57) Abstract

The present invention discloses humanized CDR-grafted antibodies which are capable of binding to the intercellular adhesion molecule ICAM-1. These antibodies are useful in treating specific and non-specific inflammation, rhinoviral infection, HIV infection, the dissemination of HIV infected cells, and asthma. In addition, the humanized antibodies disclosed can be useful in methods of diagnosing and localizing sites of inflammation and infection and tumors expressing ICAM-1.

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HUMANIZED CDR-GRAFTED ANTI-ICAM-1 ANTIBODIES, METHODS OF PREPARATION AND USAGE THEREOF

Field of the Invention

The present invention relates to a recombinant antibody molecule (RAM), and especially a CDR-grafted humanized antibody molecule (HAM), having specificity for an antigenic determinant of Intercellular Adhesion Molecule 1 (ICAM-1), to a process for its production using recombinant DNA technology and to its therapeutic use.

In the present application, the term "recombinant antibody molecule" (RAM) is used to describe an antibody produced by a process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments. The term "CDR-grafted humanized antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, the remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin, wherein the antigen binding site comprises complimentarity determining regions derived from an immunoglobulin from a non-human species grafted into appropriate human variable domain framework regions. The abbreviation "MAb" is used to indicate a monoclonal antibody.

The present invention also relates to the use of HAMs and RAMs capable of binding to ICAM-1 to inhibit intercellular adhesion of cells of granulocyte or macrophage lineage. The use of such molecules provides a method for the treatment of specific and non-specific inflammation.

The present invention also relates to RAMs and HAMs capable of

binding ICAM-1 in the treatment of viral, and particularly rhinoviral disease.

The invention also relates to therapeutic and prophylactic methods for suppressing the infection of leukocytes with HIV, and particularly with HIV-1, in an individual who is exposed to HIV or effected by HIV, and is thus in need of such suppression through the administration of RAMs and HAMs capable of binding ICAM-1 . It therefore provides a therapy for diseases, such as AIDS (Acquired Immunodeficiency Syndrome) which are caused by the HIV virus.

The invention also relates to a therapeutic method for suppressing the migration of HIV-1 infected cells from the circulatory system using RAMs and HAMs capable of binding ICAM-1 . It therefore provides a therapy for diseases, such as AIDS (Acquired Immunodeficiency Syndrome) which are caused by the HIV-1 virus.

The present invention relates to the use of RAMs and HAMs capable of binding ICAM-1 in the treatment of asthma.

Background of the Invention

A. Humanized antibodies

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise generally Y-shaped molecule having an antigen-binding site towards the free end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realization of the

potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies of defined specificity (Kohler *et al., Nature 265*:295-497 (1975)). However, most Mabs are produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice MAbs of rodent origin may not be used in a patient for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

Proposals have therefore been made for making non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanization" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanizing MABs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerization procedures are described in EPO 120694 (Celltech Limited), EPO 125023 (Genentech Inc. and City of Hope), EP-A-0171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). These patent applications generally disclose a process for preparing antibody molecules having th variable domains from a non-human MAb such as a mouse MAb and the constant domains from a human immunoglobulin. Such humanized chimeric antibodies,

however, still contain a significant proportion of non-human amino acid sequence, i.e., the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period (Begent *et al. Br. J. Cancer* 62:487 (1990)).

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted into the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates, *inter alia*, to humanized antibody molecules prepared according to this alternative approach, i.e., CDR-grafted humanized antibody molecules. Such CDR-grafted humanized antibodies are much less likely to give rise to a HAMA response than humanized chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanizing MAbs by CDR-grafting was carried out on MAbs recognizing synthetic antigens, such as the NP or NIP antigens. However, more recently examples in which a mouse MAb recognizing lysozyme and a rat MAb recognizing an antigen on human T-cells respectively were humanized by CDR-grafting have been described by Verhoeyen *et al* Verhoeyen *et al., Science* 239:1534-1536 (1988) and Riechmann *et al., Nature* 332:323-324 (1988). The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07454 (Medical Research Council).

In Riechmann *et al.* (*Nature* 332:323-324 (1988)) and Medical Research Council (WO 89/07454), it was found that transfer of the CDR regions alone (as defined by Kabat *et al.* in *Sequences of Proteins of Immunological Interest* US Department of Health and Human Services, NIH, USA (1987)) and Wu *et al., J. Exp. Med. 132*:211-250 (1970) was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann *et al.* (*Nature* 332:323-324 (1988)) found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to

obtain a CDR-grafted product having satisfactory antigen binding activity. This residue at position 27 of the heavy chain is within the structural l op adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanized antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognize more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently, Queen *et al.* (*Proc. Natl. Acad. Sci. USA 86*:10029-10033 (1989) and WO 90/07861) have described the preparation of a humanized antibody that binds to the interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximize homology with the anti-Tac MAb sequence. In addition, computer modeling was used to identify framework amino acid residues which were likely to interact with the CDR or antigen, and mouse amino acids were used at these positions in the humanized antibody.

In WO 90/07861, Queen *et al.* propose four criteria for designing humanized immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanized, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the

CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanized immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanized antibody, a humanized antibody having specificity for the p55 Tac protein of the IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanized antibody, the variable region frameworks of the human antibody Eu (Kabat *et al.*, in *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, NIH (1987)) being used as acceptor. In the resultant humanized antibody the donor CDRs were as defined by Kabat *et al.* (Kabat *et al.*, in *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, NIH (1987); and Wu *et al.*, *J. Exp. Med. 132*:211-250 (1970)) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanized anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

The preparation of CDR-grafted humanized antibody molecules has been further investigated and a hierarchy of positions within the framework of the variable regions (i.e., outside both the Kabat CDRs and structural loops of the variable regions) has been identified at which amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity: This has enabled a protocol to be established for obtaining satisfactory CDR-grafted products

which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework.

Very recently, Tempest *et al.* (*Biotechnology 9*:266-271 (1991)) have described the preparation of a reshaped human monoclonal antibody to inhibit human respiratory syncytial virus (RSV) infection *in vivo*. The reshaped antibody was prepared by grafting synthetic oligonucleotides coding for the CDRs of a murine MAb which neutralizes RSV infection by site-directed mutagenesis into DNA coding for the frameworks of a human IgG1 monoclonal antibody. However, the simple reshaped antibody in which the CDRs alone had been transferred between mouse and human antibodies had only very poor binding for RSV which was not significantly above background. In order to partially restore binding ability, it proved necessary to convert human residues to mouse residues in a framework region adjacent to CDR3 of the heavy chain.

The set of residues in the framework region which are herein disclosed as being of critical importance in the construction of functional HAMs does not coincide with the residues identified by Queen *et al.* (*Proc. Natl. Acad. Sci. USA 86*:10029-10033 (1989) and WO 90/07861). This protocol, including the identification of the critical residues, is described in detail in co-pending International Patent Application, PCT/GB 90/02017 (Celltech Limited), the disclosure of which is incorporated herein by reference, which describes, *inter alia*, the CDR-grafting of murine anti-ICAM-1 monoclonal antibodies.

B. Leukocyte Attachment and Functions

Leukocytes and granulocytes must be able to adhere to cellular substrates in order for an inflammatory response to occur and to properly defend the host against foreign invaders such as viruses, bacteria, and allergens. This fact has become evident from two converging lines of research.

The first line of research involves studies of leukocyte membrane proteins (Wallis, W.J., *et al., J. Immunol. 135*:2323-2330 (1985); Mentzer, S.J., *et al., J. Cell. Physiol. 126*:285-290 (1986); Haskard, D.O., et al., J. Immunol. 137:2901-2906 (1986); Harlan, J.M., *et al., Blood 66*:167-178 (1985)). Of particular importance to the process of cellular adhesion is a family of leukocyte membrane proteins known as the "CD18" family or complex. This family consists of three heterodimers (known as "Mac-1," "LFA-1," and "P150,90"), all of which share a common subunit (known as the ß subunit) and a unique subunit (known as the α subunit) (Springer, T.A., *et al., Immunol. Rev. 68*:111-135 (1982); Springer, T., *et al., Fed. Proc. 44*:2660-2663 (1985); Keizer, G., *et al., Eur. J. Immunol. 15*:1142-1147 (1985); Sanchez-Madrid, F., *et al., J. Exper. Med. 158*:1785-1803 (1983)).

Monoclonal antibodies against the CD18 family of leukocyte membrane proteins, by acting as antagonists of these proteins, inhibit a multitude of leukocyte adhesion dependent events *in vitro*. This includes the ability of granulocytes to aggregate in response to appropriate stimuli, the ability of granulocytes to attach to protein coated plastic, the ability of granulocytes to migrate in 2-dimensional agarose assays, and the ability of granulocytes to attach to endothelial cells.

The second line of research results from studies involving individuals, who, due to an inherited flaw in the gene encoding for the common subunit of the CD18 family of leukocyte adhesion molecules, are unable to express any of these adhesion molecules on the surfaces of their cells. Such individuals are said to suffer from "leukocyte adherence deficiency disease" ("LAD") (Anderson, D.C., *et al., Fed. Proc. 44*:2671-2677 (1985); Anderson, D.C., *et al., J. Infect. Dis. 152*:668-689 (1985)). Characteristic features of LAD patients include necrotic soft tissue lesions, impaired pus formation and wound healing, as well as abnormalities of adhesion-dependent leukocyte functions *in vitro*, and susceptibility to chronic and recurring bacterial infections. Granulocytes from these LAD patients behave in the same defective manner *in vitro* as

do their normal counterparts in the presence of anti-CD18 monoclonal antibody. That is, they are unable to perform adhesion related functions such as aggregation or attachment to endothelial cells. More importantly, however, is the observation that these patients are unable to mount a normal inflammatory response because of the inability of their granulocytes to attach to cellular substrates. Most remarkable is the observation that granulocytes from these LAD patients are unable to get to sites of inflammation such as skin infections due to their inability to attach to the endothelial cells in the blood vessels near the inflammation lesions. Such attachment is a necessary step for extravasation.

Thus, in summary, the ability of lymphocytes and granulocytes to maintain the health and viability of an animal requires that they be capable of adhering to other cells (such as endothelial cells). Granulocyte-endothelial cell adherence has been found to require cell-cell contacts which involve specific receptor molecules present on the granulocyte cell surface. These receptors enable the leukocyte to adhere to other leukocytes or to endothelial, and other non-vascular cells.

The cell surface receptor molecules of leukocytes have been found to be highly related to one another. Humans whose leukocytes lack these cell surface receptor molecules exhibit chronic and recurring infections, as well as other clinical symptoms. Inflammation reactions are mitigated when leukocytes are unable to adhere in a normal fashion due to the lack of functional adhesion molecules of the CD18 complex. Because leukocyte adhesion is involved in the process through which tissue inflammation arises, an understanding of the process of leukocyte adhesion is of significant value in defining a treatment for specific and non-specific inflammation.

Additionally, since lymphocyte adhesion is involved in the process

through which foreign body or tissue is identified and rejected, an understanding of this process is of significant value in the fields of organ transplantation, tissue grafting, allergy and oncology.

C. The Intercellular Adhesion Molecule ICAM-1 and Cellular Adhesion

The intercellular adhesion molecule ICAM-1 was first identified and partially characterized according to the procedure of Rothlein, R. *et al.* (*J. Immunol. 137*:1270-1274 (1986)), which reference is herein incorporated by reference. ICAM-1, its preparation, purification, and characteristics are disclosed in WO 90/03400 which application is herein incorporated by reference in its entirety.

ICAM-1 was initially realized as being involved in the process of cellular adhesion between endothelial cells and leukocytes. Cellular adhesion is the process through which leukocytes attach to cellular substrates, such as endothelial cells, in order to migrate from circulation to sites of ongoing inflammation, and properly defend the host against foreign invaders such as bacteria or viruses. An excellent review of the defense system is provided by Eisen, H.W., (*In: Microbiology*, 3rd Ed., Harper & Row, Philadelphia, PA (1980), pp. 290-295 and 381-418).

One of the molecules on the surface of endothelial cells which participates in the adhesion process is ICAM-1. This molecule has been shown to mediate adhesion by binding to molecules of the CD-18, CD-11/18 family of glycoproteins which are present on the cell surfaces of leukocytes (Sanchez-Madrid, F. *et al., J. Exper. Med. 158*:1785-1803 (1983); Keizer, G.D. *et al., Eur. J. Immunol. 15*:1142-1147 (1985)).

Intercellular Adhesion Molecule (ICAM-1) is an inducible cell surface glycoprotein expressed on various cell types including vascular endothelial cells, and is expressed preferentially at sites of inflammation. Since ICAM-1 is the natural binding ligand of LFA-1, ICAM-1-LFA-1

interactions play a central role in cellular adhesion, recruitment of lymphocytes to sites of inflammation and the triggering of lymphocyte functions which contribute to both specific and non-specific inflammation.

D. The Cellular Receptor for Human Rhinovirus

Abraham *et al.* (*J. Virol. 51*:340-345 (1984)) discovered that the majority of randomly selected human rhinovirus ("HRV") serotypes were able to bind to the same cellular receptor. A monoclonal antibody was subsequently developed by Colonno *et al.* (Colonno *et al., J. Cell. Biochem. Suppl. 10 (part D)*:266 (1986); Colonno *et al., J. Virol. 57*:7-12 (1986); Colonno *et al.*, European Patent Application Publication No. 169,146) which was capable of blocking attachment of HRV of the major serotype to the surfaces of endothelial cells. The endothelial cell receptor protein recognized by this antibody was isolated and found to be a 90 kd protein (Tomassini *et al., J. Virol. 58*:290-295 (1986) and later shown to be the ICAM-1 molecule (Staunton *et al., Cell 56*:849-854 (1989)).

Treatment of rhinoviral infection, especially infection by the major type human rhinovirus has been proposed using a murine monoclonal antibody directed against the viral receptor, ICAM-1 (EP 391088).

E. Infection with HIV

HIV infection is the cause of AIDS. Two major variants of HIV have been described: HIV-1 and HIV-2. HIV-1 is prevalent in North America and Europe, in contrast to HIV-2 which is prevalent only in Africa. The viruses have similar structures and encode proteins having similar function. The nucleotide and protein sequences of the genes and gene products of the two variants have been found to have about 40% homology with one another.

HIV infection is believed to occur via the binding of a viral protein (termed "gp120") to a receptor molecule (termed "CD4") present on the

surface of T4 ("T helper") lymphocytes (Schnittman, S. M. *et al., J. Immunol. 141*:4181-4186 (1988), which reference is incorporated herein by reference). The virus then enters the cell and proceeds to replicate, in a process which ultimately results in the death of the T cell. The destruction of an individual's T4 population is a direct result of HIV infection. HIV can be recovered from peripheral blood mononuclear cells and human plasma (*J. Clin. Microbiol. 26*:2371-2376 (1988); *N. Engl. J. Med. 321*:1621-1625 (1989)). Results suggest more viremia than had been previously estimated and a T-cell infection frequency as high as 1%.

The destruction of the T cells results in an impairment in the ability of the infected patient to combat opportunistic infections. Although individuals afflicted with AIDS often develop cancers, the relationship between these cancers and HIV infection is, in most cases, uncertain.

Although the mere replication of the HIV virus is lethal to infected cells, such replication is typically detected in only a small fraction of the T4 cells of an infected individual. Several lines of research have elucidated other mechanisms through which the HIV virus mediates the destruction of the T4 population.

Apart from through HIV replication, HIV infected cells can be destroyed through the action of cytotoxic, killer cells. Killer cells are normally present in humans, and serve to monitor the host and destroy any foreign cells (such as in mismatched blood transfusions or organ transplants, etc.) which may be encountered. Upon infection with HIV, T4 cells display the gp120 molecule on their cell surfaces. Killer cells recognize such T4 cells as foreign (rather than native cells), and accordingly, mediate their destruction.

HIV infection can also lead to the destruction of non-infected healthy cells. Infected cells can secrete the gp120 protein into the blood system. The free gp120 molecules can then bind to the CD4 receptors of healthy, uninfected cells. Such binding causes the cells t take on the appearance of HIV infected cells. Cytotoxic, killer cells recognize the

gp120 bound to the uninfected T4 cells, conclude that the cell is foreign, and mediate the destruction of the T4 cells.

An additional mechanism, and one of special interest to the present invention, with which HIV can cause T4 death is through the formation of "syncytia." A "syncytium" is a multinucleated giant cell, formed from the fusion of as many as several hundred T4 cells. Infection with HIV causes the infected cell to become able to fuse with other T4 cells. Such fusion partners may themselves be HIV infected, or they may be uninfected healthy cells. The syncytium cannot function and soon dies. Its death accomplishes the destruction of both HIV infected and HIV uninfected T4 cells. This process is of special interest to the present invention since it entails the direct cell-cell contact of T4 cells. The ability of HIV-infected cells to form syncytia indicates that such cells acquire a means for fusing with healthy cells. Thus, cell-cell contacts may be of fundamental importance in the process through which HIV infection is transmitted from one cell to another within an individual.

HIV infection, and especially HIV-1 infection, appears to influence cell surface expression of the leukocyte integrins and cellular adherence reactions mediated by these heterodimers (Petit, A.J., *et al., J. Clin. Invest. 79*:188 (1987); Hildreth, J.E.K., *et al., Science 244*:1075 (1989); Valentin, A., *et al., J. Immunology 144*:934-937 (1990); Rossen, R.D., *et al., Trans. Assoc. American Physicians 102*:117-130 (1989), all of which references are incorporated herein by reference). Following infection with HIV-1, homotypic aggregation of U937 cells is increased, as is cell surface expression of CD18, CD11b (Petit, A.J., *et al., J. Clin. Invest. 79*:188 (1987)). HIV-1 infected U937 cells adhere to IL-1 stimulated endothelium in greater frequency than uninfected U937 cells; this behavior can be suppressed by treating the infected cells with anti-CD18 or anti-CD11a monoclonal antibodies or by treating endothelial substrates with anti-ICAM-1 (Rossen, R.D., *et al., Trans. Assoc. American Physicians 102*:117-130 (1989)). Monoclonal antibodies to CD18 or CD11a have also been found to be able to inhibit formation of syncytia involving

phytohemagglutinin (PHA)-stimulated lymphoblastoid cells and constitutively infected, CD4-negative T cells (Hildreth, J.E.K., *et al., Science 244*:1075 (1989)). Treatment of only the virus infected cells with anti-CD18, or anti-CD11a monoclonal antibodies was found to have little effect on syncytium formation, suggesting that these antibodies principally protect uninfected target cells from infection (Hildreth, J.E.K., *et al., Science 244*:1075 (1989); Valentin, A., *et al., J. Immunology 144*:934-937 (1990)). Valentin *et al.* (Valentin, A., *et al., J. Immunology 144*:934-937 (1990)) have recently confirmed these observations by demonstrating that monoclonal antibodies specific for CD18 inhibit syncytia formed when continuous T cell lines are co-cultured with HIV-1 infected U937 cells.

Although the mechanism through which monoclonal antibodies specific for CD18 or CD11a protect susceptible cells from fusing with HIV infected cells remains unknown, and is not necessary to an appreciation of the present invention, studies with radiolabeled gp120 suggest that heterodimers containing CD18 do not provide a binding site for the virus (Valentin, A., *et al., J. Immunology 144*:934-937 (1990)). Thus, HIV infection involves cell-cell interactions, and/or viral-cell interactions which mimic such cell-cell interactions. The cell-cell interactions may result in the transport of cell-free virus or the transport of virus across endothelial barriers within the cytoplasm of infected mononuclear cells. Viral-cell interactions which mimic the cell-cell interactions may facilitate or enable free virus to attach to and/or infect healthy cells.

The present invention thus derives, in part, from the observation that HIV infection, and particularly HIV-1, infection results in increased expression of the CD11a/CD18 heterodimer, and its binding ligand, ICAM-1. This increased expression is significant in that it enhances the ability of HIV-infected T cells to adhere or aggregate with one another (i.e. to undergo "homotypic aggregation"). Since such homotypic aggregation is not observed to occur among quiescent normal leukocytes, this discovery indicates that the expression of the CD11/CD18 receptors

and/or ICAM-1 is required for such aggregation. Such adhesion permits HIV-1 to be transmitted from an infected cell to a healthy cell of an individual, and also permits or facilitates infection of healthy cells with free virus.

Since ICAM-1 plays a central role in cell-cell interactions murine monoclonal antibodies that bind to ICAM-1 have been proposed as a method of preventing HIV infection (WO 90/13281).

F. Migration of HIV Infected Cells

The migration and dissemination of leukocytes is important in protecting an individual from the consequences of infection. These processes, however, are also responsible for the migration and dissemination of viral-infected leukocytes. Of particular concern is the migration and dissemination of leukocytes infected with HIV. The migration of such cells results in the formation of extravascular foci, and may cause tumors and other abnormalities.

Histologic examination of affected organs reveals focal extravascular mononuclear cell infiltrates. Attempts to identify virus-infected cells in such infiltrates in the central nervous system have revealed the presence of HIV-1 infected cells. These studies have shown that HIV-1 resides primarily in monocytes and macrophages, and other cells of this lineage (R.T. Johnson, *et al. FASEB J.* 2:2970 (1988); M.H. Stoler *et al., J. Amer. Med. Assn.* 256:2360 (1986); S. Gartner *et al. J. Amer. Med. Assn.* 256:2365 (1986); S. Gartner *et al. Science* 233:215 (1986)).

The mechanisms which stimulate formation of extravascular infiltrates of HIV-1-infected monocytoid cells have not previously been well defined. The mechanisms may involve either the transport of cell-free virus or the transport of virus across endothelial barriers within the cytoplasm of infected mononuclear cells.

Since infection with HIV-1 stimulates cell surface expression of molecules which facilitate adherence of leukocytes to vascular endothelial cells and the translocation of leukocytes from the blood to extravascular tissue sites (C.W. Smith *et al., J. Clin. Invest. 82*:1746 (1988), herein incorporated by reference) it has been proposed to use antibodies which inhibit cellular migration to prevent the dissemination of HIV infected cells (WO 90/13316).

G. Asthma: Clinical Characteristics

Asthma is a heterogeneous family of diseases. It is characterized by a hyper-responsiveness of the tracheobronchi to stimuli (McFadden, E.R. *et al.*, In: *Harrison's Principles of Internal Medicine*, 10th Ed., Petersdorf, R.G. *et al.*, Eds., McGraw-Hill, NY (1983), pages 1512-1519); Kay, A.B., *Allergy and Inflammation*, Academic Press, NY (1987); which references are incorporated herein by reference). Clinically, asthma is manifested by the extensive narrowing of the tracheobronchi, by thick tenacious secretions, by paroxysms of dyspnea, cough, and wheezing. Although the relative contribution of each of these conditions is unknown, the net result is an increase in airway resistance, hyperinflation of the lungs and thorax, abnormal distribution of ventilation and pulmonary blood flow. The disease is manifested in episodic periods of acute symptoms interspersed between symptom-free periods. The acute episodes result in hypoxia, and can be fatal. Approximately 3% of the general world population suffers from the disease.

Two types of asthma have been described: allergic asthma and idiosyncratic asthma. Allergic asthma is usually associated with a heritable allergic disease, such as rhinitis, urticaria, eczema, etc. The condition is characterized by positive wheal-and-flare reactions to intradermal injections of airborne antigens (such as pollen, environmental or occupational pollutants, etc.), and increased serum levels of IgE. The development of allergic asthma appears to be causally related to the

pres nc of IgE antibodies in many patients. Asthma patients who do not exhibit the above-described characteristics are considered to have idiosyncratic asthma.

Allergic asthma is believed to be dependent upon an IgE response controlled by T and B lymphocytes and activated by the interaction of airborne antigen with mast cell-bound pre-formed IgE molecules. The antigenic encounter must occur at concentrations sufficient to lead to IgE production for a prolonged period of time in order to sensitize an individual. Once sensitized, an asthma patient may exhibit symptoms in response to extremely low levels of antigen.

Asthma symptoms may be exacerbated by the presence and level of the triggering antigen, environmental factors, occupational factors, physical exertion, and emotional stress.

Asthma may be treated with methylxanthines (such as theophylline), beta-adrenergic agonists (such as catecholamines, resorcinols, saligenins, and ephedrine), glucocorticoids (such as hydrocortisone), inhibitors of mast cell degranulation (i.e. chromones such as cromolyn sodium) and anticholinergics (such as atropine).

Asthma is believed to involve an influx of eosinophils ("eosinophilia") into the tissues of the lung (Frigas, E. *et al., J. Allergy Clin. Immunol. 77*:527-537 (1986), which reference is incorporated herein by reference).

Insight into the immunological basis of asthma has been gained from bronchoalveolar lavage studies (Godard, P. *et al., J. Allergy Clin. Immunol. 70*:88 (1982)), and studies of respiratory smooth muscle denuded of epithelium (Flavahan, N.A. *et al., J. Appl. Physiol. 58*:834 (1985); Barnes, P. J. *et al., Br. J. Pharmacol. 86*:685 (1985)). Although these studies have not led to the elucidation of the mechanism underlying the immunology of asthma, they have led to the development of a generally accepted hypothesis concerning the immunological etiology of the disease (see, Frigas, E. *et al., J. Allergy Clin. Immunol. 77*:527-537 (1986)).

The hallmarks of the pathology of asthma are a massive infiltration of the lung parenchyma by eosinophils and the destruction of mucociliary capacity. The "eosinophil hypothesis" suggests that eosinophils are attracted to the bronchus in order to neutralize harmful mediators released by the mast cells of the lung. According to the hypothesis eosinophils are attracted to the bronchi where they degranulate to release cytotoxic molecules. Upon degranulation, eosinophils release enzymes such as histaminase, arylsulfatase and phospholipase D which enzymatically neutralize the harmful mediators of the mast cell. These molecules also promote the destruction of the mucociliary apparatus, and thus prevent the clearing of the bronchial secretions, and contribute to the lung damage characteristic of asthma.

Since asthma involves the migration of cells, it has been proposed to use antibodies which inhibit this migration to mitigate the effects of allergens in a subject (WO 90/10453).

H. Conclusion

It has been previously proposed; to treat leucocyte-mediated inflammation by administering *inter alia* an anti-ICAM-1 antibody to patients suffering from such inflammation (see EP-0289949 and EP-0314863), to treat viral infection by administering *inter alia* an anti-ICAM-1 antibody to patients suffering from such infection (EP-391088), to prevent the infection of a subject with HIV by administering *inter alia* an anti-ICAM-1 antibody (WO 90/13281), to prevent the dissemination of HIV infected cells by administering *inter alia* an anti-ICAM-1 antibody (WO 90/13316), and to administer *inter alia* an anti-ICAM-1 antibody to mitigate the effects of allergens (WO 90/10453).

EP-289949 describes the preparation of a murine monoclonal (R6-5-D6) having specificity for ICAM-1 which is the preferred antibody for the above referenced therapies. Samples of R6-5-D6 have been deposited with the American Type Culture Collection as deposit ATCC HB9580 on

30th Octob r 1987. R6-5-D6 has been deposited with the ATCC under the provisions of Rule 28(4) of the EPC.

Currently available anti-ICAM-1 MAbs, which are the basis of the above described methods of treatment, are murine MAbs and as a result are likely to cause a significant HAMA response if administered in repeat doses to human patients. It would be highly desirable to diminish or abolish this undesirable HAMA response by suitable humanization or other appropriate recombinant DNA manipulation of these potentially highly useful antibodies and thus extend and enlarge their use. It would also be desirable to apply the techniques of recombinant DNA technology to these antibodies to prepare anti-ICAM-1 RAMs in general.

We have now prepared anti-ICAM-1 RAMs and CDR-grafted humanized antibody molecules derived from murine MAbs.

SUMMARY OF THE INVENTION

The present invention provides a method of constructing a recombinant antibody molecule (RAM). Specifically the RAMs of the present invention comprises the antigen binding regions derived from the heavy and/or light chain variable regions of an anti-ICAM-1 antibody.

The present invention provides a method for the construction of CDR-grafted humanized antibody molecules (HAM). Specifically the HAMs of the present invention have specificity for ICAM-1 and have an antigen binding site wherein at least one or more of the complementarity determining regions (CDRs) of the variable domains are derived from a non-human anti-ICAM-1 antibody.

The invention further pertains to the HAMs and RAMs of the present invention which are detectably labeled.

The invention additionally includes a recombinant DNA molecule capable of expressing the RAMs or HAMs of the present invention.

The invention further includes a host cell capable of producing HAMs or RAMs of the present invention when transformed by the recombinant DNA molecules disclosed herein.

The invention additionally includes diagnostic and therapeutic uses for the HAMs and RAMs of the present invention.

The invention further provides a method for treating inflammation resulting from a response of the specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress the inflammation; wherein the anti-inflammatory agent is a HAM or RAM of monoclonal antibody capable of binding to ICAM-1.

The invention further provides a method for treating non-specific inflammation in humans, and other mammals.

In detail, the invention includes a method for treating inflammation resulting from a response of the specific and non-specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an anti-inflammatory agent, capable of binding to an ICAM-1, in an amount sufficient to suppress the inflammation; wherein the anti-inflammatory agent is a HAM or RAM capable of binding to ICAM-1.

The invention further includes the above-described method for treating inflammation wherein the inflammation is associated with a condition selected from the group consisting of: adult respiratory distress syndrome; multiple organ injury syndrome secondary to septicemia; multiple organ injury syndrome secondary to trauma; reperfusion injury of myocardial or other tissues; acute glomerulonephritis; reactive arthritis; dermatosis with acute inflammatory components; acute purulent meningitis or other central nervous system inflammatory disorders; thermal injury; hemodialysis; leukapheresis; ulcerative colitis; Crohn's disease; necrotizing enterocolitis; granulocyte transfusion associated syndrome; and cytokine-induced toxicity.

The invention further provides a method of suppressing the metastasis of a hematopoietic tumor cell, the cell requiring a functional member of the LFA-1 family for migration, wherein said method comprises providing to a patient in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress the metastasis; wherein the anti-inflammatory agent is a HAM or RAM capable of binding to ICAM-1.

The invention further provides a method of suppressing the growth of an ICAM-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress the growth, the toxin being derivatized to one of the HAMs or RAMs of the present invention.

The invention further provides a method of diagnosing the presence and location of an inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having the inflammation which comprises:

(a) administering to the subject a composition containing a detectably labeled HAM or RAM capable of identifying a cell which expresses ICAM-1, and

(b) detecting the binding ligand.

The invention additionally provides a method of diagnosing the presence and location of an inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having the inflammation which comprises:

(a) incubating a sample of tissue of the subject with a composition containing a detectably labeled HAM or RAM capable of identifying a cell which expresses ICAM-1, and

(b) detecting the binding ligand.

The invention further provides a method of diagnosing the presence and location of an ICAM-1-expressing tumor cell in a mammalian subject suspected of having such a cell, which comprises:

(a) administering to the subject a composition containing a detectably labeled HAM or RAM capable of binding to ICAM-1, and

(b) detecting the binding ligand.

The invention further provides a method of diagnosing the presence and location of an ICAM-1-expressing tumor cell in a mammalian subject suspected of having such a cell, which comprises:

(a) incubating a sample of tissue of the subject with a composition containing a detectably labeled HAM or RAM capable of binding ICAM-1, and

(b) detecting the binding ligand.

The invention additionally includes a pharmaceutical composition comprising:

(a) an anti-inflammatory agent consisting of a RAM or HAM capable of binding to ICAM-1, and

(b) at least one immunosuppressive agent selected from the group consisting of: dexamethasone, azathioprine and cyclosporin A.

The present invention also relates to the use of HAMs and RAMs capable of binding ICAM-1 in anti-viral therapy.

In detail, the invention provides a method for treating viral infection, wherein said virus binds to ICAM-1 in an individual in need of such treatment, wherein the method comprises providing to the individual an amount of a HAM or RAM capable of binding ICAM-1 sufficient to suppress viral infection.

The invention further provides a method for suppressing the infection of leukocytes with HIV, which comprises administering to a patient exposed to or effected by HIV, an effective amount of an HIV-1 infection suppression agent, the agent being a RAM or HAM capable of binding to ICAM-1.

The invention further concerns the embodiment of the above method wherein the HIV is HIV-1.

The invention further provides a method for suppressing the extravascular migration of a virally infected leukocyte in a patient having

such a leukocyte, which comprises administering to the patient an effective amount of a HAM or RAM capable of impairing the ability of said leukocyte to bind to ICAM-1.

The invention further comprises the embodiment of the above-described method wherein the virally infected leukocytes are infected with HIV.

The invention further comprises the embodiment of the above-described method wherein the agent is a HAM or RAM capable of binding to ICAM-1.

The invention further provides a method for treating asthma in a patient which comprises providing to the patient an effective therapeutic amount of a HAM or RAM capable of binding to ICAM-1

The invention further concerns the embodiment of the above described methods wherein the HAM or RAM capable of binding to ICAM-1 is derived from the murine monoclonal antibody R6-5-6D.

BRIEF DESCRIPTION OF THE FIGURES

The present invention is now described, by way of example only, with reference to the accompanying diagrams, Figures 1-17, in which:

Figure 1 shows the cDNA sequence for the 5' untranslated region, signal sequence, variable region and part constant region together with corresponding amino acid sequence for the R6-5-D6 murine MAb light chain;

Figure 2 shows similar cDNA and amino acid sequence for the R6-5-D6 murine mab heavy chain;

Figure 3 shows a plasmid diagram of plasmid expression vector PEE6 hCMV;

Figure 4 shows plasmid diagrams indicating the strategy for construction of light chain expression plasmid pAL5;

Figure 5 shows plasmid diagrams indicating the strategy for construction of heavy chain expression plasmid pAL6;

Figure 6 shows a graph giving results of a competition assay comprising binding of recombinant and murine R6-5-D6 and a control MAb UPC10;

Figure 7 shows the amino acid sequence using the single letter code, of the variable region for the light chains of the grafted genes gL221 and gL221A. Underlined are the amino acids derived from the murine sequence.

Figure 8 shows the oligonucleotide pairs used to build the variable region gene for the gL221 construct.

Figure 9 shows an outline diagram of the process of construction of the plasmid pBJ1 which is an expression vector capable of directing the expression and secretion of the gL221 light chain in a suitable eukaryotic host cell. The 408 bp BstBI-Sp1I fragment is cloned into pE1081 at the unique BstB1 and Sp1I sites and generates a full light chain gene in which the humanized variable region gene is fused directly to the human kappa constant region gene. The resultant light chain protein has the correct V-C junction sequence.

Figure 10 shows the oligonucleotide pairs used to build the variable region gene for the gL221A construct.

Figure 11 shows the amino acid sequence, using the single letter code, of the variable region for the heavy chains of the murine anti-ICAM-1 antibody, the humanized gH341, gH341A, gH341B and gH341D chains and for comparison the EU heavy variable region sequence.

In gH341, gH341A, gH341B and gH341D the murine sequence included in the gene designs are noted by underlining.

Figure 12 shows the oligonucleotide pairs used to build the variable region gene for the gH341 construct.

Figure 13 shows an outline of the process of construction of the gH341B gene using a Polymerase Chain Reaction (PCR) process for the introduction of a single amino acid substitution.

Figure 14 shows the oligonucleotides used to build the XhoI-AapaI gene fragment which was used to construct the gH341D gene.

Figure 15 and Figure 16 shows the results of COS cell expression in which various gene combinations noted on the figures were used to produce novel antibody. The yield of antibody was calculated by ELISA as described in the Methods Section and used to tabulate the measured binding of the antibody to the ICAM-1 antigen on JY cells.

Figure 17 shows the results of a competition binding assay with chimeric and CDR-grafted antibodies.

Brief Description of the Preferred Embodiments

A. Humanized Antibodies

The first embodiment of the present invention provides a RAM comprising antigen binding regions derived from the heavy and/or light chain variable regions of an anti-ICAM-1 antibody.

Typically the anti-ICAM-1 antibody is a rodent MAb.

A second embodiment of the present invention provides a CDR-grafted humanized antibody molecule (HAM) having specificity for the ICAM-1 antigen and having an antigen binding site wherein at least the complementarity determining regions (CDRs) of the variable domains are derived from a non-human (e.g., rodent) anti-ICAM-1 antibody. Typically the HAM is prepared by recombinant DNA technology.

In the present invention, the anti-ICAM-1 binding region typically comprises at least one CDR from the anti-ICAM-1 antibody. Usually the anti-ICAM-1 antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs and any combination of any of these.

The HAMs of the present invention characteristically do not include humanized chimeric antibody molecules in which the antigen binding site comprises a complete heavy and/or light chain variable domain derived from an antibody of desired antigen binding specificity. The HAM is characteristically a CDR-grafted antibody.

The HAMs of the present invention preferably comprise CDR-grafted antibody as defined in co-pending International Patent Application PCT/GB 90/02017 (Celltech Limited).

Preferably the CDRs of the light chain correspond to the Kabat CDRs at CDR1 (positions 24-34) and CDR2 (positions 50-56) and to the structural loop residues in (positions 91-96) or Kabat CDR residues (positions 89-97) in CDR3. In addition the light chain may have mouse residues at one or more of positions 1, 2 and/or 3, 46, 47, 49, 60, 70, 84, 85 and 87 and preferably has non-human residues at at least positions 46 and 47.

The HAM heavy chain preferably has non-human (eg mouse) residues at positions 23 and/or 24 and 71 and/or 73. Additionally, the heavy chain may have non-human residues at one, some or all of positions 48 and/or 49, 69, 76 and/or 78, 80, 88 and/or 91 and 6. Preferably also, the CDRs of the heavy chain correspond to the Kabat CDR at CDR2 (positions 50-65), the structural loop residues at CDR3 (positions 95-100) and a composite of both the Kabat and structural loop residues at CDR1 (positions 24-35); for example, when the human variable region framework used is KOL. Alternatively, the CDRs of the heavy chain may comprise non-human (eg mouse) residues at positions 26 to 35 for CDR1, positions 50 to 56 for CDR2 and positions 94 to 100 for CDR3; f r

example, when the human variable region framework used in EU. In addition EU has a particularly idiosyncratic J region between residues 103 to 113 and it may be useful to include the murine amino acids, or a consensus human J region or a suitable combination of both at residues 103 to 108 inclusive.

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering (Kabat *et al.*, in *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, NIH (1987); and Wu *et al.*, *J. Exp. Med.* *132*:211-250 (1970)). Thus, the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering, corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen *et al.* (*Proc. Natl. Acad. Sci. USA* *86*:10029-10033 (1989) and WO 90/07861) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The RAMs or HAMs of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as the Fab or $(Fab')_2$ fragment; a light chain or heavy chain monomer or dimer, or a single chain antibody, e.g., a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other recombinant or CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

The remaining immunoglobulin-derived parts of the RAM or HAM may be derived from any suitable human immunoglobulin. Appropriate variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of human framework used is of the same/similar class/type as the donor antibody. Advantageously, the framework is chosen to maximize/optimize homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. Examples of human frameworks which may be used to construct CDR-grafted HAMs are KOL, NEWM, REI EU, LAY and POM (Kabat *et al.*, in *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, NIH (1987); and Wu *et al.*, *J. Exp. Med. 132*:211-250 (1970)) and the like; for instance, KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also human constant region domains of the HAM may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used especially of the IgG1 and IgG3 isotopes, when the HAM is intended for therapeutic purposes and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the HAM is intended for therapeutic purposes and antibody effector functions are not required e.g. for simple blocking of ICAM-1/LFA-1 interactions. With humanized anti-ICAM-1 antibodies selection of the IgG1 isotype has been shown to result in the highest avidity binding (see our co-pending International Patent Application of even date herewith which relates to humanized chimeric anti-ICAM-1 antibodies).

The remainder of the HAM need not comprise only protein sequences from the human immunoglobulin. For instance, a gene may be constructed in which a DNA sequence encoding part of a human

immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

Alternatively, the RAM or HAM of the present invention may be a "chemical derivative" of the RAM or HAM. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980). "Toxin-derivatized" molecules constitute a special class of "chemical derivatives." A "toxin-derivatized" molecule is a molecule (such as ICAM-1 or an antibody) which contains a toxin moiety. The binding of such a molecule to a cell brings the toxin moiety into close proximity with the cell and thereby promotes cell death. Any suitable toxin moiety may be employed; however, it is preferable to employ toxins such as, for example, the ricin toxin, the diphtheria toxin, radioisotopic toxins, membrane-channel-forming toxins, etc. Procedures for coupling such moieties to a molecule are well known in the art. Alternatively the RAM or HAM can be attached to a macrocycle, for chelating a heavy metal atom.

Alternatively, the procedures of recombinant DNA technology may be used to produce a "chemical derivative" of the RAM or HAM in which the Fc fragment or CH3 domain of a complete antibody molecule has been replaced by or has attached thereto by peptide linkage a functional non-immunoglobulin protein such as an enzyme or toxin molecule.

The invention also provides recombinant DNA processes for the preparation of the HAMs of the invention.

According to a third embodiment the present invention a process for producing an anti-ICAM-1 humanized antibody molecule which process is provided which comprises:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain wherein at least one or more of the CDRs of the variable domain are derived from a non-human (rodent) anti-ICAM-1 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light or heavy chain wherein at least one or more of the CDRs of the variable domain are derived from a non-human (rodent) anti-ICAM-1 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the HAM.

In addition to the CDR's specific variable region framework residues may correspond to non-human (e.g. mouse) residues as described above in relation to preferred embodiments of the second embodiment of the invention. It will be appreciated, however, that this embodiment of the invention, as for previous embodiments, does not encompass processes for the production of chimeric antibodies, i.e., antibodies in which substantially the complete heavy and/or light chain variable domain is derived from a non-human (rodent) anti-ICAM-1 antibody.

The cell line may be transfected with two vectors, the first vector containing an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the

coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

Alternatively, a single vector may be used, the vector including the DNA sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially genomic DNA. Most preferably, the heavy or light chain encoding sequence comprises a fusion of cDNA and genomic DNA.

Thus, the present invention also includes cloning and expression vectors and transfected cell lines used in the process of the invention.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known *per se* and form no part of the invention. Such methods are shown, for instance, in Maniatis *et al.*, *Molecular Cloning*, Cold Spring Harbor, New York (1982); and Primrose and Old, *Principles of Gene Manipulation*, Blackwell, Oxford (1980).

The anti-ICAM-1 antibodies of the invention include all anti-ICAM-1 specificities. Typically, however, the antibodies have specificity for antigenic epitopes of ICAM-1 which when bound by the antibody block, inhibit or otherwise modify ICAM-1/LFA-1 and or ICAM-1/Mac-1 interactions. Preferably the antibodies have specificity for the same or similar ICAM-1 antigenic epitopes as the R6-5-D6 etc. antibodies. Most especially the antibodies are derived from the R6-5-D6 antibody.

B. Therapeutics and Diagnosis

The present invention also includes therapeutic and diagnostic compositions containing the RAM or HAM of the invention and uses of such compositions in therapy and diagnosis.

The therapeutic uses to which the products of the anti-ICAM-1 invention may be put include any of the therapeutic uses to which anti-ICAM-1 antibodies may be put including for example any or all of the therapeutic uses described in EP-0289949, EP-0314863, and corresponding applications.

1. Anti-Inflammatory Agents

A. Specific Inflammation

Monoclonal antibodies to members of the CD 18 or CD-11/18 complex inhibit many adhesion dependent functions of leukocytes including binding to endothelium (Haskard, D., *et al., J. Immunol. 137*:2901-2906 (1986)), homotypic adhesions (Rothlein, R., *et al., J. Exp. Med. 163*:1132-1149 (1986)), antigen and mitogen induced proliferation of lymphocytes (Davignon, D., *et al., Proc. Natl. Acad. Sci., USA 78*:4535-4539 (1981)), antibody formation (Fischer, A., *et al., J. Immunol. 136*:3198-3203 (1986)), and effector functions of all leukocytes such as lytic activity of cytotoxic T cells (Krensky, A.M., *et al., J. Immunol. 132*:2180-2182 (1984)), macrophages (Strassman, G., *et al., J. Immunol. 136*:4328-4333 (1986)), and all cells involved in antibody-dependent cellular cytotoxicity reactions (Kohl, S., *et al., J. Immunol. 133*:2972-2978 (1984)). In all of the above functions, the antibodies inhibit the ability of the leukocyte to adhere to the appropriate cellular substrate which in turn inhibits the final outcome. Although both polyclonal and monoclonal antibodies may be employed to inhibit these function, the present invention provides an improvement through the use of a HAM or RAM derived from an anti-ICAM-1 monoclonal antibody.

As discussed previously, the binding of ICAM-1 molecules to the members of LFA-1 family of molecules is of central importance in cellular adhesion. Through the process of adhesion, lymphocytes ar capable of continually monitoring an animal for the presence of foreign antigens. Although these processes are normally desirable, they are also the cause

of organ transplant rejection, tissue graft rejection and many autoimmune diseases. Hence, any means capable of attenuating or inhibiting cellular adhesion would be highly desirable in recipients of organ transplants (e.g. kidney), tissue grafts or autoimmune patients.

A HAM or RAM capable of binding to ICAM-1 is highly suitable as anti-inflammatory agents in a mammalian subject. Significantly, such an agent differs from general anti-inflammatory agents and non-humanized antibodies in that they are capable of selectively inhibiting adhesion, do not offer other side effects such as nephrotoxicity which are found with conventional agents, and limit the amount of HAMA associated with the use of murine MAbs. A HAM or RAM capable of binding to ICAM-1 can therefore be used to prevent organ (e.g. kidney) or tissue rejection, or modify autoimmune responses without the fear of such side effects, in the mammalian subject.

Importantly, the use of a HAM or RAM capable of recognizing ICAM-1 may permit one to perform organ transplants even between individuals having HLA mismatch.

In the forth embodiment of the present invention a method for suppressing specific inflammation is provided wherein said method comprises providing to recipient subjects in need of such a treatment an amount of one of the HAMs or RAMs of the present invention sufficient to suppress inflammation. An amount is said to be sufficient to "suppress" inflammation if the dosage, route of administration, etc. of the agent are sufficient to attenuate or prevent inflammation.

The HAM or RAM may be administered either alone or in combination with one or more additional immunosuppressive agents (especially to a recipient of an organ or tissue transplant). The administration of such a composition may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the immunosuppressive composition is provided in advance of any inflammatory response or symptom (for example, prior to, at, or shortly after) the time of an organ or tissue transplant but in advance of any

symptoms of organ rejection). The prophylactic administration of the composition serves to prevent or attenuate any subsequent inflammatory response (such as, for example, rejection of a transplanted organ or tissue, etc.). When provided therapeutically, the immunosuppressive composition is provided at (or shortly after) the onset of a symptom of actual inflammation (such as, for example, organ or tissue rejection). The therapeutic administration of the composition serves to attenuate any actual inflammation (such as, for example, the rejection of a transplanted organ or tissue).

The anti-inflammatory agents of the present invention may, thus, be provided either prior to the onset of inflammation (so as to suppress an anticipated inflammation) or after the initiation of inflammation.

Since ICAM-1 molecules are expressed mostly at sites of inflammation, such as those sites involved in delayed type hypersensitivity reaction, antibodies (especially HAMs and RAMs derived from anti-ICAM-1 monoclonal antibodies) capable of binding to ICAM-1 molecules have therapeutic potential in the attenuation or elimination of such reactions. This potential therapeutic use may be exploited in either of two manners. First, a composition containing a HAM or RAM capable of binding to ICAM-1 may be administered to a patient experiencing delayed type hypersensitivity reaction. For example, such compositions might be provided to a individual who had been in contact with antigens such as poison ivy, poison oak, etc.

In a sixth embodiment, a HAM or RAM of the present invention is administered to a patient in conjunction with an antigen in order to prevent a subsequent inflammatory reaction. Thus, the additional administration of an antigen with an ICAM-1-binding HAM or RAM may temporarily tolerize an individual to subsequent presentation of that antigen.

Since LAD patients that lack LFA-1 do not mount an inflammatory response, it is believed that antagonism of LFA-1's natural ligand, ICAM-1, will also inhibit an inflammatory response. The ability

of antibodies against ICAM-1 to inhibit inflammation provides the basis for their therapeutic use in the treatment of chronic inflammatory diseases and autoimmune diseases such as lupus erythematosus, autoimmune thyroiditis, experimental allergic encephalomyelitis (EAE), multiple sclerosis, some forms of diabetes Reynaud's syndrome, rheumatoid arthritis, etc. Such antibodies may also be employed as a therapy in the treatment of psoriasis. In general, a HAM or RAM capable of binding ICAM-1 may be employed in the treatment of those diseases currently treatable through steroid therapy.

B. Non-specific Inflammation

The present invention derives from the discovery that ICAM-1 on endothelial cells binds to the members of the CD18 family of molecules on granulocytes responsible for mediating granulocyte-endothelial cell adhesion and that antagonists of ICAM-1 are capable of inhibiting such adhesion. Such inhibition provides a means for treating general, non-specific tissue inflammation.

Since cellular adhesion is required in order that leukocytes may migrate to sites of non-specific inflammation and/or carry out various effector functions contributing to the inflammation, agents which inhibit cellular adhesion will attenuate or prevent this inflammation. A "non-specific defense system reaction" is a response mediated by leukocytes incapable of immunological memory. Such cells include granulocytes and macrophages. As used herein, inflammation is said to result from a response of the non-specific defense system, if the inflammation is caused by, mediated by, or associated with a reaction of the non-specific defense system. Examples of inflammation which result, at least in part, from a reaction of the non-specific defense system include inflammation associated with conditions such as: adult respiratory distress syndrome (ARDS) or multiple organ injury syndromes secondary to septicemia or trauma; reperfusion injury of myocardial or other tissues; acute

glomerulonephritis; reactive arthritis; dermatoses with acute inflammatory components; acute purulent meningitis or other central nervous system inflammatory disorders (e.g. stroke); thermal injury; hemodialysis; leukapheresis; ulcerative colitis; Crohn's disease; necrotizing enterocolitis; granulocyte transfusion associated syndromes; and cytokine-induced toxicity.

In a fifth embodiment of the present invention a method of treating non-specific inflammation is provided wherein said method comprises providing to a subject in need of such treatment an affective amount of a HAM or RAM of the present invention.

2. Diagnostic and Prognostic Applications

Since ICAM-1 is expressed mostly at sites of inflammation, a HAM or RAM capable of binding ICAM-1 may be employed as a means of imaging or visualizing the sites of infection and inflammation in a patient.

In an eighth embodiment of the present invention, a HAM or RAM is detectably labeled, through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.) fluorescent labels, paramagnetic atoms, etc and are provided to a patient to localize the site of infection or inflammation. Procedures for accomplishing such labeling are well known to the art. Clinical application of antibodies in diagnostic imaging are reviewed by Grossman, H.B., *Urol. Clin. North Amer. 13*:465-474 (1986)), Unger, E.C. *et al., Invest. Radiol. 20*:693-700 (1985)), and Khaw, B.A. *et al., Science 209*:295-297(1980)).

The detection of foci of such detectably labeled antibodies is indicative of a site of inflammation or tumor development. In one embodiment, this examination for inflammation is done by removing samples of tissue, including blood cells, and incubating such samples in the presence of th detectably labeled antibodies. In a preferred embodiment, this technique is done in a non-invasive manner through the use of magnetic imaging, fluorography, etc. Such a diagnostic test may be

employed in monitoring organ transplant recipients for early signs of potential tissue rejection. Such assays may also be conducted in efforts to determine an individual's predilection to rheumatoid arthritis or other chronic inflammatory diseases.

3. Adjunct to the Introduction of Antigenic Material Administered for Therapeutic or Diagnostic Purposes

Immune responses to therapeutic or diagnostic agents such as, for example, bovine insulin, interferon, tissue-type plasminogen activator or murine monoclonal antibodies substantially impair the therapeutic or diagnostic value of such agents, and can, in fact, causes diseases such as serum sickness. Such a situation can be remedied through the use of the HAMs and RAMs of the present invention. In this embodiment, such antibodies would be administered in combination with the therapeutic or diagnostic agent.

In a ninth embodiment of the present invention the addition of an effective amount of a HAM or RAM with specificity to ICAM-1 is administer to a subject in order to prevent the recipient from recognizing the agent, and therefore prevent the recipient from initiating an immune response against it. The absence of such an immune response results in the ability of the patient to receive additional administrations of the therapeutic or diagnostic agent.

4. Anti-viral usage of HAMs and RAMs

Another aspect of the present invention relates to the discovery of that ICAM-1 is the cellular receptor of certain viruses, and is thus required in order for the virus to adhere to and infect human cells (Greve, J.M. *et al., Cell 56*:839-847 (1989); Staunton, D.E. *et al., Cell 56*:849-853 (1989), both of which references are incorporated by reference herein in their entirety). In particular, rhinoviruses, and especially

rhinoviruses of the major serotype have been found to be capable f mediating their infection through their capacity to bind to the ICAM-1 molecules present on cell surfaces.

The tenth embodiment of the present invention is directed toward the use of HAMs and RAMs capable of binding ICAM-1 to treat viral infection. Because such antibodies are capable of blocking the ICAM-1 of endothelial cells for viral attachment, their administration to a recipient individual results in the decrease in receptors available for viral binding, and thus decreases the percentage of viruses which attach and infect the cells of an individual.

ICAM-1 has the ability to interact with and bind to viruses, and in particular, rhinoviruses of the major serotype within the genus Picornaviridae, group A coxsackieviruses (Colonno, R.J. *et al., J. virol. 57*:7-12 (1986)) and Mengo viruses (Rossmann, M.G. *et al., Virol. 164*:373-382 (1988)). This interaction is mediated by ICAM-1 amino acid residues which are present in domain 1 of the ICAM-1 molecule. Such interactions are assisted, however, by contributions from amino acids present in domains 2 and 3 of ICAM-1. Thus, among the preferred RAMs and HAMs of this embodiment are antibodies capable of binding to domains 1, 2, and 3 of ICAM-1. More preferred are HAMs and RAMs capable of binding to domains 1 and 2 of ICAM-1. Most preferred are HAMs and RAMs capable of binding domain 1 of ICAM-1.

The administration of the anti-viral agents of the present invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the anti-viral agent is provided in advance of any symptom of viral infection (for example, prior to, at, or shortly after the time of infection, but in advance of any symptoms of such infection). The prophylactic administration of the agent serves to prevent or attenuate any subsequent viral infection, or to reduce the possibility that such infection will be contagious to others.

When provided therapeutically, the anti-viral agent is provided at (or shortly after) the onset of a symptom of actual viral infection (such as,

for example, nasal congestion, fever, etc. The therapeutic administration of the agent serves to attenuate any actual viral infection.

The anti-viral agents of the present invention may, thus, be provided either prior to the onset of viral infection (so as to suppress an anticipated infection) or after the initiation of such infection.

5. Suppression of HIV Infection and the Prevention of the Dissemination of HIV Infected Cells.

The eleventh embodiment of present invention provides a method for suppressing the infection of HIV, which comprises administering to an HIV-infected individual an effective amount of an HIV infection suppression agent. Although the invention is particularly concerned with a method for the suppression of HIV-1 infection, it is to be understood that the method may be applied to any HIV-1 variant (such as, for example, HIV-2) which may infect cells in a way which may be suppressed by the agents of the present invention. Such variants are the equivalents of HIV-1 for the purposes of the present invention.

One aspect of the present invention derives from the recognition that expression of LFA-1 and, in some cases, ICAM-1, stimulated by HIV infection, promotes cell-to-cell adherence reactions that can increase the contact time of infected with uninfected cells, facilitating transfer of virus from infected to uninfected cells. Thus, HAMs and RAMs derived from capable of binding ICAM-1 are able to suppress infection by HIV, and, in particular, by HIV-1.

One means through which molecules which bind to ICAM-1 may suppress HIV infection is by impairing the ability of the ICAM-1 expressed by HIV-infected cells to bind to the CD11/CD18 receptors of a healthy T cell. In order to impair the ability of a cell to bind to the CD11a/CD18 receptor, or to the ICAM-1 ligand molecule, it is possible to employ either HAMs and RAMs capable of binding to ICAM-1.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to achieve a suppression of HIV infection. An amount is said to be sufficient to "suppress" HIV infection if the dosage, route of administration, etc. of the agent are sufficient to attenuate or prevent such HIV infection. The agents are to be provided to patients who are exposed to, or effected by HIV infection.

The HAMs and RAMs of the present invention may be for either a "prophylactic" or "therapeutic" purpose in the treatment of HIV infection. When provided prophylactically, the antibody is provided in advance of any symptom of viral infection (for example, prior to, at, or shortly after) the time of such infection, but in advance of any symptoms of such infection). The prophylactic administration of the antibody serves to prevent or attenuate any subsequent HIV infection. When provided therapeutically, the antibody is provided at (or shortly after) the detection of virally infected cells. The therapeutic administration of the antibody serves to attenuate any additional HIV infection.

The agents of the present invention may, thus, be provided either prior to the onset of viral infection (so as to suppress the anticipated HIV infection) or after the actual detection of such virally infected cells (to suppress further infection).

In particular, the invention provides an improved therapy for AIDS, and an enhanced means for suppressing HIV infection, and particularly HIV-1 infection, which comprises the co-administration of:

(I) ICAM-1, a soluble ICAM-1 derivative, CD11 (either CD11a, CD11b, or CD11c), a soluble CD11 derivative, CD18, a soluble CD18 derivative, or a CD11/CD18 heterodimer, or a soluble derivative of a CD11/CD18 heterodimer and/or

(II) a HAM or RAM capable of binding to ICAM-1 with

(III) cell or particle associated CD4 or a soluble derivative of CD4 and/or

(IV) a molecule (preferably an antibody or antibody fragment) capable of binding to CD4.

In the twelfth embodiment of the present invention also a method for suppressing the migration of HIV-infected cells is provided wherein said method comprises administering an effective amount of an anti-migration agent to an HIV-infected individual.

The anti-migration agents of the present invention include any HAM or RAM capable of impairing the ability of an HIV-infected T cell to bind to ICAM-1. HAMs and RAMs which bind to ICAM-1 will suppress migration by impairing the ability of the ICAM-1 expressed by HIV-infected T cells to bind to cells expressing a CD11/CD18 receptor. In order to impair the ability of a cell to bind to the CD11a/CD18 receptor it is possible to employ a HAM or RAM capable of binding to ICAM-1.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to suppress the migration of HIV (or other virally) infected T cells. An amount is said to be sufficient to "suppress" migration of T cells if the dosage, route of administration, etc. of the agent are sufficient to attenuate or prevent such migration.

The administration of such compound(s) may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the HAM or RAM is provided in advance of any symptom of viral infection (for example, prior to, at, or shortly after) the time of such infection, but in advance of any symptoms of such infection). The prophylactic administration of the HAM or RAM serves to prevent or attenuate any subsequent migration of virally infected T cells. When provided therapeutically, the HAM or RAM is provided at (or shortly after) the detection of virally infected T cells. The therapeutic administration of the antibody serves to attenuate any additional migration of such T cells.

The HAMs and RAMs of the present invention may, thus, be provided either prior to the onset f viral infection (so as to suppress the anticipated migration of infected T cells) or after the actual detection of such virally infected cells.

6. Treatment of Asthma

In the thirteenth embodiment of the present invention a HAM and RAM capable of binding to ICAM-1 is used in the treatment of asthma.

The therapeutic effects of the anti-asthma agents of the present invention may be obtained by providing such agents to a patient by any suitable means (i.e. intravenously, intramuscularly, subcutaneously, enterally, or parenterally). It is preferred to administer the agents of the present invention intranasally as by nasal spray, swab, etc. It is especially preferred to administer such agents by oral inhalation, or via an oral spray or oral aerosol. When administering agents by injection, the administration may be by continuous infusion, or by single or multiple boluses.

The anti-asthma agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to lessen or attenuate the severity, extent or duration of the asthma symptoms.

The HAMs and RAMs of the present invention may be administered either alone or in combination with one or more additional anti-asthma agents (such as methylxanthines (such as theophylline), beta-adrenergic agonists (such as catecholamines, resorcinols, saligenins, and ephedrine), glucocorticoids (such as hydrocortisone), chromones (such as cromolyn sodium) and anticholinergics (such as atropine), in order to decrease the amount of such agents needed to treat the asthma symptoms.

The administration of the HAMs or RAMs of the present invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the HAMs or RAMs are provided in advance of any asthma symptom. The prophylactic administration of the

HAMs or RAMs serves to prevent or attenuate any subsequent asthmatic response. When provided therapeutically, the HAMs or RAMs are provided at (or shortly after) the onset of a symptom of asthma. The therapeutic administration of the antibody serves to attenuate any actual asthmatic episode. The antibodies of the present invention may, thus, be provided either prior to the onset of an anticipated asthmatic episode (so as to attenuate the anticipated severity, duration or extent of the episode) or after the initiation of the episode.

C. Administration of the Compositions of the Present Invention

The therapeutic effects of HAMs or RAMs capable of binding ICAM-1 may be obtained by providing to a patient an effective amount of a HAM or RAM which is substantially free of natural contaminants.

The HAMs and RAMs of the present invention disclosed herein are said to be "substantially free of natural contaminants" if preparations which contain them are substantially free of materials with which these products are normally and naturally found.

The present invention extends to HAMs and RAMs which may be produced either by an animal, or by tissue culture, or recombinant DNA means.

In providing a patient with a HAM or RAM, the dosage of administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc. In general, it is desirable to provide the recipient with a dosage of antibody which is in the range of from about 1 pg/kg to 10 mg/kg (body weight of patient), although a lower or higher dosage may be administered.

A HAM or RAM capable of binding to ICAM-1 may be administered to patients intravenously, intramuscularly, subcutaneously, enterally, topically inhaled, intranasally or parenterally. When

administering an antibody, the administration may be by continuous administration, or by single or multiple boluses.

A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

The HAMs and RAMs of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these antibodies are combined in a mixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in Remington's Pharmaceutical Sciences (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of a HAM or RAM together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Controlled release preparations may be achieved through the use of polymers to complex or absorb the HAM or RAM. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate the HAM or RAM into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating the HAM or RAM into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by

coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly-(methylmethacylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980).

MATERIAL METHODS

1. Incoming Cells

Hybridoma cell line R6-5-D6 was provided by Boehringer Ingelheim Pharmaceuticals Inc. (Lot No. R6-5-D6 - E9-B2 0-29-86) and was grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain murine IgG2a/kappa antibody. Cell culture supernatant was examined and confirmed to contain the antibody R6-5-D6.

2. Molecular Biology Procedures

Basic molecular biology procedures were as Maniatis *et al.* (1982) (Maniatis *et al., Molecular Cloning,* Cold Spring Harbor, New York (1982)) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger *et al.* (1977) (Sanger *et al., Proc. Natl. Acad. Sci. USA 74*:5463-5467 (1977)) and the Amersham International Plc sequencing handbook. COS cell expression and metabolic labelling studies were as described in Whittle *et al.* (1987) (Whittle *et al., Prot. Eng. 1, 6*:499-505 (1987)). Chinese Hamster Ovary (CHO) transfections and cell culture were performed as described in Gorman (1988) (Gorman, C., *DNA Cloning 2:*143-190 *ed.*(1988)) and Bebbington and Hentschel (1988) (Bebbington *et al., DNA Cloning 3*:163-188 *ed.* (1988)).

3. Research Assays

3.1. Assay for Secreted Antibody Light Chain

Supernatants from CHO cell lines were assayed for secreted light chain, after transfection with light chain expression vectors, as the first step in the development of stable cell lines producing whole chimeric antibody. The procedure was as follows:

96 well microtitre plates were coated with $F(ab')_2$ goat anti-human kappa light chain. The plates were washed with water and samples added and incubated for one hour at room temperature. The plates were washed and $F(ab')_2$ goat anti-human $F(ab')_2$ Horse radish peroxidase (HRPO) conjugate was then added and incubated for a further hour. Enzyme substrate was then added to reveal the reaction.

3.2. Assembly Assays

Assembly assays were performed on supernatants from transfected COS cells and from transfected CHO cells to determine the amount of intact IgG present.

3.2.1 COS and CHO Cells transfected with mouse genes

The assembly assay for intact mouse IgG in cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with $F(ab')_2$ goat anti-mouse IgG Fc. The plates were washed in water and samples added and incubated for 1 hour at room temperature. The plates were washed and $F(ab')_2$ goat anti-mouse IgG $F(ab')_2$ (HRPO conjugated) was then added. Enzyme substrate was then added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.2.2 COS and CHO Cells transfected with Chimeric Genes

The assembly assay for intact humanized anti-ICAM-1 in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with $F(ab')_2$ goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added and incubated for 1 hour at room temperature. The plates were washed and $F(ab')_2$ goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was then added to reveal the reaction. Chimeric B72.3 (Bodmer *et al.*, Published International Patent Application WO 89/01783) (IgG4) and later purified anti-ICAM-1 (IgG4, IgG2, and IgG1) were used as standards. The use of a monoclonal anti-kappa chain in this assay allows the amount of CDR-grafted antibodies to be read from the standards.

3.3. Assay for Antigen Binding Activity

3.3.1 Direct Binding

Material from COS and CHO cell supernatants and purified chimeric antibodies were assayed for anti-ICAM-1 antigen binding activity onto ICAM-1 positive cells in a direct assay. The procedure was as follows:

JY cells (a human B lymphoblastoid cell line which constitutively expresses ICAM-1 on the cell surface) were maintained in culture. Monolayers of JY cells were fixed onto 96 well ELISA plates using poly-L-lysie and paraformaldehyde. Samples were added to the monolayers and incubated for 1 hour at room temperature. The plates were washed gently using PBS. $F(ab;)_2$ goat anti-human IgG Fc (HPO conjugated) or $F(ab')_2$ goat anti-mouse IgG Fc (HRPO conjugated) which was added as appropriate for humanized or mouse samples. Enzyme substrate was then added to reveal the reaction. The negative control for the cell-based assay was chimeric B72.3 (IgG4) or pooled, purified human IgG2 and IgG4 (Chemicon). The positive control was murine R6.5.D6 MAb or chimeric anti-ICAM-1 antibodies.

3.3.2 Competition Binding

Monolayers of JY cells were prepared as in 3.3.1. Antibody samples were added and incubated overnight at 4 C. Biotinylated anti-ICAM-1 was added to all the wells. The mixture was left at room temperature for 2 hours. The plates were washed and streptavidin-HRPO was added. After further incubation enzyme substrate was added to reveal the reaction.

Results

4. cDNA Library Construction

4.1. mRNA Preparation and cDNA Synthesis

Cells were grown as described in Section 1 and 1.4 x 10^9 cells harvested and mRNA extracted using the guanidinium/LiC1 extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoRI linkers added for cloning.

4.2. Library Construction

The cDNA library was ligated to pSP64 vector DNA which had been EcoRI cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoRI/CIP). The ligation was used to transform high transformation efficiency Escherichia coli HB101 (24 E. coli HB101) from Bethesda Research Lbs (BRL) in the case of the light chain and *E. coli* LM1035 prepared by electroporation (Dower *et al., Nucl. Acids Res. 16*:6127 (1988)) in the case of the heavy chain. cDNA libraries were prepared. 11600 colonies were screened for the light chain and 25000 colonies were screened for the heavy chain.

5. Screening

E. coli colonies positive for either heavy or light chain probes were identified either by oligonucleotide screening using the oligonucleotide:

5'TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, or by using a 980 bp BamHI-EcoRI restriction fragment of a previously isolated mouse IgG2a constant region clone. 6 light chain and 10 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and DNA inserts of a size capable of containing a full length cDNA were sequenced.

6. DNA Sequencing

DNA sequence for the 5' untranslated regions, signal sequences, variable and constant regions and 3' untranslated regions of full length cDNAs were obtained and are given in Figure 1 for the light chain and Figure 2 for the heavy chain.

7. Construction of cDNA Expression Vectors

Celltech expression vectors are based on the plasmid pEE6 hCMV as shown in Figure 3 (Bebbington, C.R., Published International Patent Application WO 89/01036). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamHI cassettes in the unique BamHI site of pEE6 hCMV. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoRI sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised as EcoRI fragments and cloned into either EE6-hCMV-neo for the light chain (Figure 4) and into EE6-hCMV-gpt for the heavy chain (Figure 5).

8. Expression of cDNAs in COS Cells

Plasmids pAL5 (Figure 4) and pAL6 (Figure 5) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to JY cells (Figure 6). Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. Construction of CDR-grafted genes

Analysis of human antibody sequences which might be used as receptor frameworks suggested that the antibody EU would be a suitable candidate (see Kabat *et al.* 1987)

9.1. Light Chain Sequence

Figure 7 shows the amino acid sequence of two variable regions in which the mouse framework regions have been replaced by the analogous sequences from the EU light chain. In gL221 the only mouse sequences used are residues 24-34, 50-56 and 89-97 inclusive. In gL221A several framework residues have been left as mouse. This was done after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residue numbers are 2, 3, 49, 60, 70, 84, 85 and 87 and are noted in the Figure by underlining.

Figure 8 shows the oligonucleotide pairs used to build the variable region gene for the gL221 construct. Oligonucleotides were chemically phosphorylated during synthesis. 20 pmole of complementary oligonucleotides were paired and heated to 65°C and allowed to anneal by cooling to room temperature. All of the pairs were then mixed and ligated overnight using T4 DNA ligase. The terminal restriction sites

were exposed by cleavage of the ligation products with the enzymes BstBI and Sp1I. The required 408 bp fragment was isolated by preparative agarose gel electrophoresis and ligated into pE1081 which is a vector, similar to pEE6 hCMV described above, containing the hCMV promoter and the human kappa constant region in a configuration such that insertion of the variable region sequence on a BstBI-Sp1I fragment will allow efficient expression and secretion of full length light chain sequence in suitable eukaryotic cells, e.g. Cos-1 cells.

Candidate clones were confirmed by DNA sequencing. One clone pBJ2 was used for expression studies. Figure 9 shows a diagram of the construction procedure. The gL221A gene was assembled in a similar manner to give a plasmid pBJ1.

Figure 10 shows the oligonucleotide pairs used to build the variable region gene for the gL221A construct.

9.2 Heavy Chain Sequence

Figure 11 shows the amino acid sequence of heavy chain variable regions in which the mouse framework regions have been replaced by analogous sequences from the EU heavy chain. EU heavy chain has a particularly idiosyncratic J region and the murine sequence from the anti-ICAM antibody R6-5-D6 in fact more closely resembles the normal human sequence motif than does EU. Therefore the murine sequence between residues 103 to 108 inclusive (numbered according to EU index, Kabat *et al., in Sequences of Proteins of Immunological Interest* US Department of Health and Human Services, NIH, USA (1987) were incorporated into the gene design.

In gH341 the mouse sequences from residues 26-35, 50-56 and 94-100B (numbered according to EU index Kabat *et al., in Sequences of Proteins of Immunological Interest* US Department of Health and Human Services, NIH, USA (1987) were included, as well as those mentioned earlier. In gH341A at amino acids 24 and 73 the mouse residues were used. In gH341B at amino acids 24, 48 and 73 mouse residues were used.

In gH341D at amino acids 24, 48, 69, 71, 73, 80, 88, and 91 the mouse residues were used. These extra mouse residues were included to accommodate decisions on the likely contribution of these residues to domain integrity and the correct positioning of the antigen binding region.

Figure 12 shows the oligonucleotide pairs used to build the variable region gene for the gH341 construct. The gene was assembled as described in 9.1 for gL221. To isolate the required gene fragment restriction enzymes HindIII and ApaI were used. The 440 bp gene fragment was cloned into the HindIII and ApaI sites of pE1004 (a vector similar to pEE6 hCMV described above) which is a vector containing the hCMV promoter and the human IgG4 constant region in a configuration such that introduction of the variable region sequence will allow efficient expression and secretion of full length heavy chain sequence, when co-expressed with a vector capable of producing a suitable light chain, in a eukaryotic cell, e.g. COS-1 cells. The vector also encodes the hypoxanthine guanine phosphoribosyl transferase gene (gpt gene) to allow for the formation of stable cell lines in, for example, Chinese Hamster Ovary (CHO) cells. Candidate clones were confirmed by DNA sequencing. One clone, pJA192 was used for expression studies. The gH341A gene was assembled in a similar manner to give pJA195.

The residues 24 and 73 investigated in the gH341A gene in JA192 are separated to form two further genes, gH341A1 and gH341A2, using a restriction site, Xho1, which lies between the two codons in the gH341A gene. By isolation of HindIII to Xho1 fragments from pJA192 (gH341 vector) and pJA195 (gH341A vector) and swapping of gene fragments the derivative vectors containing the gH341A1 and gH341A2 genes can be constructed. This allows an examination of the relative effects of these amino acids no net acidity of resultant antibody.

The gH341B gene was prepared from gH341A in pJA195 by a Polymerase Chain Reaction (PCR) mutagenesis procedure in which the codon for amino acid 48 was altered such that the resultant amino acid changed from the human residue, methionine, to the mouse amino acid

isoleucine. The process for the construction of gH341B is outlined in Figure 13. A candidate clone pAL19 was confirmed by DNA sequencing and used for expression studies.

The gH341D gene was assembled by oligonucleotide assembly of the 3' half of the variable region and was cloned into pJA195 (gH341A) as a 294 bp XhoI-ApaI fragment. Figure 13 shows the oligonucleotides used to assemble this gene fragment. The resultant vector was termed pAL20.

As an alternative to the use of EU as heavy chain acceptor, an alternative acceptor framework could be used. For example, the antibody KOL has been shown to be a useful acceptor.

By examination of the sequence and consideration of those framework residues likely to be involved in domain integrity and correct positioning of the antigen binding region, an amino acid sequence for the variable region can be designed. The gene, gH341A (KOL) has murine sequence at regions 36-35, 50-62, 64-65 and 95-100b inclusive (Kabat *et al., in Sequences of Proteins of Immunological Interest* US Department of Health and Human Services, NIH, USA (1987)) and also at amino acid locations 24, 71 and 73. The oligonucleotides required to assemble the gene are shown in Figure 14. 10 pmole of each oligonucleotide is mixed in a 100 μl final volume of buffer. The mixture is subjected to 30 cycles of temperature adjustment of to 95°C for 1 minute, 55°C for 1 minute and 72°C 1 minute with 0.5 units of Taq Polymerase. The required fragment can be recovered after HindIII and ApaI digestion of the reaction products and can be cloned into pE1004 for expression studies.

10. Expression of CDR-grafted antibodies

Expression vectors containing CDR-grafted light or heavy chain genes were co-transfected into COS cells as described. Combinations of light and heavy chains were performed and included the use of chimeric light (cL) and chimeric IgG4 heavy chains (cH) as control chains.

(Constructs of cL and cH genes are described in our co-pending UK Patent Application of even filing date herewith.)

Figure 15 shows a series of transfections in which the gL221 and gL221A genes were co-transfected with cH, and the gH341, gH341A, gH341B, gH341D genes were co-transfected with cL. Comparison was made to the cL cH combination.

It can be seen for the gL221/cH and gL221A/cH combinations that both gave an antibody with binding activity apparently equivalent to that of the chimeric antibody. In the case of the grafted heavy/chimeric light combinations increasing the amount of mouse sequence outside of the CDRs apparently leads to antibody with improved binding characteristics.

Figure 16 shows a comparison of the gH341D gene co-expressed with either gL221 or gL221A. It can be seen that the binding of the antibodies relative to the chimeric antibody is about 75% and 50% respectively for the gL221A/gH341D and gL221/gH341D combinations.

Thus significant binding activity is retained by introducing the mouse framework residues in the gH341 gene.

Figure 17 shows the result of a competition assay in which the IgG4 gL221A gH341D antibody successfully competes for binding to ICAM-1 on JY cells against the parent mouse antibody R6.5. Competitive ability is about 10% that of the chimeric IgG4 antibody.

CLAIMS

1. A recombinant antibody molecule comprising antigen binding regions derived from the heavy and/or light chain variable regions of an anti-ICAM-1 antibody.

2. A CDR-grafted humanized antibody molecule having specificity for ICAM-1 and having an antigen binding site wherein at least one of the complementarity determining regions of the variable domains are derived from a non-human anti-ICAM-1 antibody.

3. A CDR-grafted antibody light chain according to claim 2 comprising non-human CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 91-96 (CDR3).

4. A CDR-grafted light chain according to claim 2 or 3, comprising non-human residues at one or more of positions 1, 2 and/or 3, 46, 47, 49, 60, 70, 84, 85 and 87.

5. A CDR-grafted light chain according to claim 4 comprising non-human residues at at least positions 46 and 47.

6. A CDR-grafted antibody heavy chain according to claim 2 comprising non-human CDRs at positions 24-35 (CDR1), 50-65 (CDR2) and 95-100 (CDR3).

7. A CDR-grafted antibody heavy chain according to claim 2 comprising non-human CDRs at positions 26-35 (CDR1), 50-65 (CDR2) and 94-100 (CDR3).

8. A CDR-grafted heavy chain according to claim 2, 6 or 7 comprising non-human residues at positions 23 and/or 24 and 71 and/or 73.

9. A CDR-grafted heavy chain according to claim 8 additionally comprising non-human residues at one, some or all of positions 6, 48 and/or 49, 69, 76 and/or 78, 80, 88 and/or 91.

10. A recombinant antibody molecule or a CDR-grafted humanized antibody molecule comprising at least one antibody light chain according to any one of claims 1-5 and at least one antibody heavy chain according to any one of claims 1, 2 or 6-9.

11. A recombinant antibody molecule or a CDR-grafted humanized antibody molecule wherein said antibody is derived from the R6-5-D6 murine monoclonal antibody.

12. DNA coding an antibody heavy chain or light chain according to any one of claims 1-11.

13. A vector comprising DNA according to claims 10 or 11.

14. An expression vector comprising DNA coding for an antibody light chain according to any one of claims 1-5 in operative combination with DNA coding for an antibody heavy chain according to any one of claims 1, 2 or 6-9.

15. Host cells transformed with a vector according to claim 12 or 13.

16. A process for the production of an anti-ICAM-1 HAM comprising:

(a) producing an expression vector comprising an operon having a DNA sequence which encodes an antibody heavy or light chain wherein at least one of the CDRs of the variable domain are derived from a non-human (rodent) anti-ICAM-1 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(b) producing an expression vector comprising an operon having a DNA sequence which encodes a complementary antibody light or heavy chain wherein at least one of the CDRs of the variable domain are derived from a rodent (non-human) anti-ICAM-1 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(c) transfecting a host cell with each vector; and

(d) culturing the transfected cell line to produce the HAM.

17. A therapeutic composition comprising an antibody molecule, or a fragment thereof, according to any one of claims 1-10 in combination with a pharmaceutically acceptable diluent, excipient or carrier.

18. A diagnostic composition comprising an antibody molecule, or a fragment thereof, according to any one of claims 1-11 in a detectably labelled form.

19. A method of treatment comprising administering an effective amount of an antibody product according to any one of claims 1-11 to a human or animal subject.

20. A method for treating inflammation resulting from a r sponse of the specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation,

wher in said anti-inflammatory agent is a HAM or RAM capable of binding ICAM-1.

21. The method of claim 20, wherein said HAM or RAM is one or more of the antibodies of claims 1-11.

22. The method of claim 20, wherein said inflammation is a delayed type hypersensitivity reaction.

23. The method of claim 20, wherein said inflammation is a symptom of psoriasis.

24. The method of claim 20, wherein said inflammation is a symptom of an autoimmune disease.

25. The method of claim 24, wherein said autoimmune disease is selected from the group consisting of Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis, rheumatoid arthritis and lupus erythematosus.

26. The method of claim 20, wherein said inflammation is in response to organ transplant rejection.

27. The method of claim 26, wherein said organ transplant is a kidney transplant.

28. The method of claim 20, wherein said inflammation is in response to tissue graft rejection.

29. The method of claims 20 which additionally comprises the administration of an agent selected from the group consisting of: an antibody capable of binding to LFA-1; a functional derivative of said

antibody, said functional derivative being capable of binding to LFA-1; and a non-immunoglobulin antagonist of LFA-1.

30. A method for treating inflammation resulting from a response of the non-specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation, wherein said anti-inflammatory agent is a HAM or RAM capable of binding ICAM-1.

31. The method of claims 30 wherein said inflammation is associated with a condition selected from the group consisting of: adult respiratory distress syndrome; multiple organ injury syndrome secondary to septicemia; multiple organ injury syndrome secondary to trauma; reperfusion injury of tissue; acute glomerulonephritis; reactive arthritis; dermatosis with acute inflammatory components; a central nervous system inflammatory disorder e.g. stroke; thermal injury; hemodialysis; leukapheresis; ulcerative colitis; Crohn's disease; necrotizing enterocolitis; granulocyte transfusion associated syndrome; and cytokine-induced toxicity.

32. The method of claims 30 or 31 wherein said HAM or RAM is one or more of the antibodies of claims 1-11.

33. A method of suppressing the metastasis of a hematopoietic tumor cell, said cell requiring a functional member of the LFA-1 family for migration, which method comprises providing to a patient in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said metastasis, wherein said anti-inflammatory agent is a chimeric antibody capable of binding ICAM-1.

34. The method of claim 33, wherein said HAM or RAM capable of binding to ICAM-1 is selected from the group of antibodies of claims 1-11.

35. A method of suppressing the growth of an ICAM-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress said growth, said toxin consists of a toxin-derivatized HAM or RAM capable of binding to ICAM-1.

36. A method for treating viral infection in an individual in need of such treatment, wherein said method comprises providing to said individual an amount of a HAM or RAM capable of binding ICAM-1 sufficient to suppress viral infection.

37. The method of claim 36, wherein said virus is a rhinovirus of the major serotype within the genus Picornaviridae, a group A coxsackievirus, or a Mengo virus.

38. The method of claim 37 wherein said virus is a rhinovirus of the major serotype.

39. The method of any of claims 36-38 wherein said HAM or RAM is at least one of the antibodies of claims 1-11.

40. A method for suppressing the infection of leukocytes with HIV, which comprises administering to a patient exposed to or infected by HIV, an effective amount of an HIV-1 infection suppression agent, said agent being a HAM or RAM capable of binding to ICAM-1.

41. The method of claim 40 wherein said HIV is HIV-1.

42. The method of any one of claims 40 or 41, wherein said HAM or RAM is at least one of the antibodies of claims 1-11.

43. A method for suppressing the extravascular migration of a virally infected leukocyte in a patient having such a leukocyte, which comprises administering to said patient an effective amount of an anti-migration agent, said agent being a HAM or RAM capable of impairing the ability of said leukocyte to bind to ICAM-1.

44. The method of claim 43, wherein said virally infected cells are infected with HIV.

45. The method of claims 43 or 44, wherein said HAM or RAM is at least one of the antibodies of claims 1-11.

46. A method for treating asthma in an individual in need of such treatment, wherein said method comprises providing to said individual an amount of a HAM or RAM capable of binding ICAM-1 sufficient to suppress asthma.

47. The method of claim 46 wherein said HAM or RAM is at least one of the antibodies of claims 1-11.

48. The method of any one of claims 19-47 wherein said humanized HAM or RAM is administered by enteral means, parenteral means, topical means, inhalation means or intranasal means.

49. The method of claim 48 wherein said humanized HAM or RAM is administered prophylactically.

50. The method of claim 48 wherein said humanized HAM or RAM is administered therapeutically.

51. The method of claims 48, 49 or 50 wherein said parenteral means is intramuscular, intravenous or subcutaneous.

52. A pharmaceutical composition comprising the anti-inflammatory agent of any one of claims 1-11 in combination with a pharmaceutically acceptable carrier.

53. The pharmaceutical composition of claim 52 in combination with at least one other immunosuppressive agent.

54. A method of diagnosing an ICAM-1-expressing tumor cell in a mammalian subject which comprises:

(a) administering to said subject a composition containing a detectably labeled HAM or RAM capable of binding to ICAM-1, and

(b) detecting said HAM or RAM bound to said ICAM-1.

55. A method of diagnosing inflammation in a mammalian subject which comprises:

(a) incubating a sample of tissue of said subject with a composition containing a detectably labeled chimeric antibody capable of binding to a cell which expresses ICAM-1, and

(b) detecting said chimeric antibody bound to said cell.

DNA Sequence of Anti-Icam Light chain with translation.

```
       GAATTCCGCCTCCTCAAAATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATT
    45 ----+---------+---------+---------+---------+---------+---------+---- 104
       CTTAAGGCGGAGGAGTTTTACTTCAACGGACAATCCGACAACCACGACTACAAGACCTAA
c                       M  K  L  P  V  R  L  L  V  L  M  F  W  I   -

       CCTGATTCCAGCAGTGATGTTGTGATGACCCAAAGTCCACTCTCCCTGCCTGTCAGTCTT
   105 ----+---------+---------+---------+---------+---------+---------+---- 164
       GGACTAAGGTCGTCACTACAACACTACTGGGTTTCAGGTGAGAGGGACGGACAGTCAGAA
c      P  D  S  S  S  D  V  V  M  T  Q  S  P  L  S  L  P  V  S  L   -
                     *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

       GGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACAGCAATGGAAAT
   165 ----+---------+---------+---------+---------+---------+---------+---- 224
       CCTCTAGTTCGGAGGTAGAGAACGTCTAGATCAGTCTCGGAACATGTGTCGTTACCTTTA
c      G  D  Q  A  S  I  S  C  R  S  S  Q  S  L  V  H  S  N  G  N   -

       AACTATTTACATTGGTACCTGCAGAAGTCAGGCCAGGCTCCAAAGCTCCTGATCTACAAA
   225 ----+---------+---------+---------+---------+---------+---------+---- 284
       TTGATAAATGTAACCATGGACGTCTTCAGTCCGGTCCGAGGTTTCGAGGACTAGATGTTT
c      N  Y  L  H  W  Y  L  Q  K  S  G  Q  A  P  K  L  L  I  Y  K   -

       GTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT
   285 ----+---------+---------+---------+---------+---------+---------+---- 344
       CAAAGGTTGGCTAAAAGACCCCAGGGTCTGTCCAAGTCACCGTCACCTAGTCCCTGTCTA
c      V  S  N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D   -

       TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTCTGCTCTCAA
   345 ----+---------+---------+---------+---------+---------+---------+---- 404
       AAGTGTGAGTTCTAGTCGTCTCACCTCCGACTCCTAGACCCTCAAATAAAGACGAGAGTT
c      F  T  L  K  I  S  R  V  E  A  E  D  L  G  V  Y  F  C  S  Q   -

       AGTACACATGTTCCTCTCACGTTCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGAT
   405 ----+---------+---------+---------+---------+---------+---------+---- 464
       TCATGTGTACAAGGAGAGTGCAAGCCTCCCCCCTGGTTCGACCTTTATTTTGCCCGACTA
c      S  T  H  V  P  L  T  F  G  G  G  T  K  L  E  I  K  R  A  D   -

       GCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGxGCC
   465 ----+---------+---------+---------+---------+---------+---------+---- 524
       CGACGTGGTTGACATAGGTAGAAGGGTGGTAGGTCACTCGTCAATTGTAGACCTCCxCGG
c      A  A  P  T  V  S  I  F  P  P  S  S  E  Q  L  T  S  G  G  A   -

       TCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAG
   525 ----+---------+---------+---------+---------+---------+-- 581
       AGTCAGCACACGAAGAACTTGTTGAAGATGGGGTTTCTGTAGTTACAGTTCACCTTC
c      S  V  V  C  F  L  N  N  F  Y  P  K  D  I  N  V  K  W  K   -
```

KEY:

The protein sequence overlined is the leader sequence.

The protein sequences underlined are the CDR's.

The protein sequence underlined and overlined is part of the constant domain.

The starred (*) sequence was confirmed from protein sequencing.

Figure 1

DNA Sequence of Anti-Icam Heavy chain with translation.

```
      GAATTCCGTCACAGGAGTACACAGGGCATTGCCATGGGTTGGAGCTGTATCATCTTCTTT
    1 ---------+---------+---------+---------+---------+---------+ 60
      CTTAAGGCAGTGTCCTCATGTGTCCCGTAACGGTACCCAACCTCGACATAGTAGAAGAAA
a                                     M  G  W  S  C  I  I  F  F  -

      CTGGTAGCAACAGCTACAGGTGTGCACTCCCAGGTCCAGCTGCAGCAGTCTGGGCCTGAG
   61 ---------+---------+---------+---------+---------+---------+ 120
      GACCATCGTTGTCGATGTCCACACGTGAGGGTCCAGGTCGACGTCGTCAGACCCGGACTC
a     L  V  A  T  A  T  G  V  H  S  Q  V  Q  L  Q  Q  S  G  P  E  -

      CTGGTGAGGCCTGGGGTCTCAGTGAAGATTTCCTGCAAGGGTTCCGGCTACACATTCATT
  121 ---------+---------+---------+---------+---------+---------+ 180
      GACCACTCCGGACCCCAGAGTCACTTCTAAAGGACGTTCCCAAGGCCGATGTGTAAGTAA
a     L  V  R  P  G  V  S  V  K  I  S  C  K  G  S  G  Y  T  F  I  -

      GATTATGCTATACACTGGGTGAAGGAGAGTCATGCAAAGAGTCTAGAGTGGATTGGAGTT
  181 ---------+---------+---------+---------+---------+---------+ 240
      CTAATACGATATGTGACCCACTTCCTCTCAGTACGTTTCTCAGATCTCACCTAACCTCAA
a     D  Y  A  I  H  W  V  K  E  S  H  A  K  S  L  E  W  I  G  Y  -

      ATTAGTGCTTACTCTGGTGACACAAACTACAACCAGAAGTTTAAGGGCAAGGCCACAATG
  241 ---------+---------+---------+---------+---------+---------+ 300
      TAATCACGAATGAGACCACTGTGTTTGATGTTGGTCTTCAAATTCCCGTTCCGGTGTTAC
a     I  S  A  Y  S  G  D  T  N  Y  N  Q  K  F  K  G  K  A  T  M  -

      ACTGTTGACAAATCCTCCAACACAGCCTATTTGGAACTTGCCAGATTGACATCTGAGGAT
  301 ---------+---------+---------+---------+---------+---------+ 360
      TGACAACTGTTTAGGAGGTTGTGTCGGATAAACCTTGAACGGTCTAACTGTAGACTCCTA
a     T  V  D  K  S  S  N  T  A  Y  L  E  L  A  R  L  T  S  E  D  -

      TCTGCCATCTATTACTGTGCAAGAGGGGGATGGTTACTACTCTCCTTTGACTACTGGGGC
  361 ---------+---------+---------+---------+---------+---------+ 420
      AGACGGTAGATAATGACACGTTCTCCCCCTACCAATGATGAGAGGAAACTGATGACCCCG
a     S  A  I  Y  Y  C  A  R  G  G  W  L  L  L  S  F  D  Y  W  G  -

      CAAGGCACCACTCTCACAGTCTCCTCAGCCAAAACAACAGCCCCATCGGTCTATCCACTG
  421 ---------+---------+---------+---------+---------+---------+ 480
      GTTCCGTGGTGAGAGTGTCAGAGGAGTCGGTTTTGTTGTCGGGGTAGCCAGATAGGTGAC
a     Q  G  T  T  L  T  V  S  S  A  K  T  T  A  P  S  V  Y  P  L  -

      GCCCCTGTGTGTGGAGATACAACTGGCTCCTCGGTGACTCTAGG
  481 ---------+---------+---------+---------+--- 524
      CGGGGACACACACCTCTATGTTGACCGAGGAGCCACTGAGATCC
a     A  P  V  C  G  D  T  T  G  S  S  V  T  L  ?  -
```

KEY:

The protein sequence in overlined is the leader sequence.

The protein sequences underlined are the CDR's.

The protein sequence underlined and overlined is part of the constant domain.

Figure 2

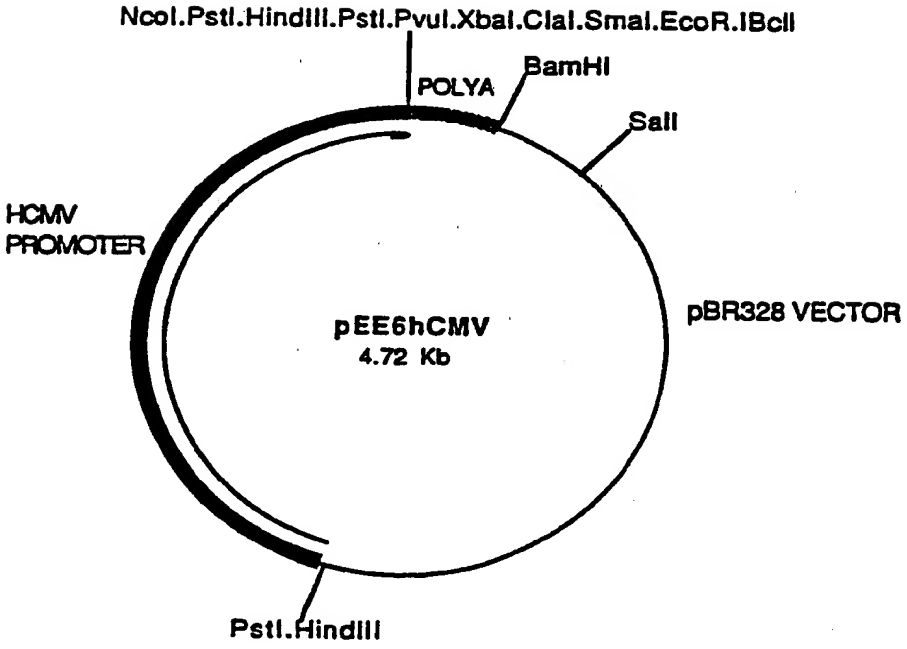


FIGURE 3

Figure 3 shows a map for the EE6hCMV expression vector used in this study
Only necessary sites are shown.

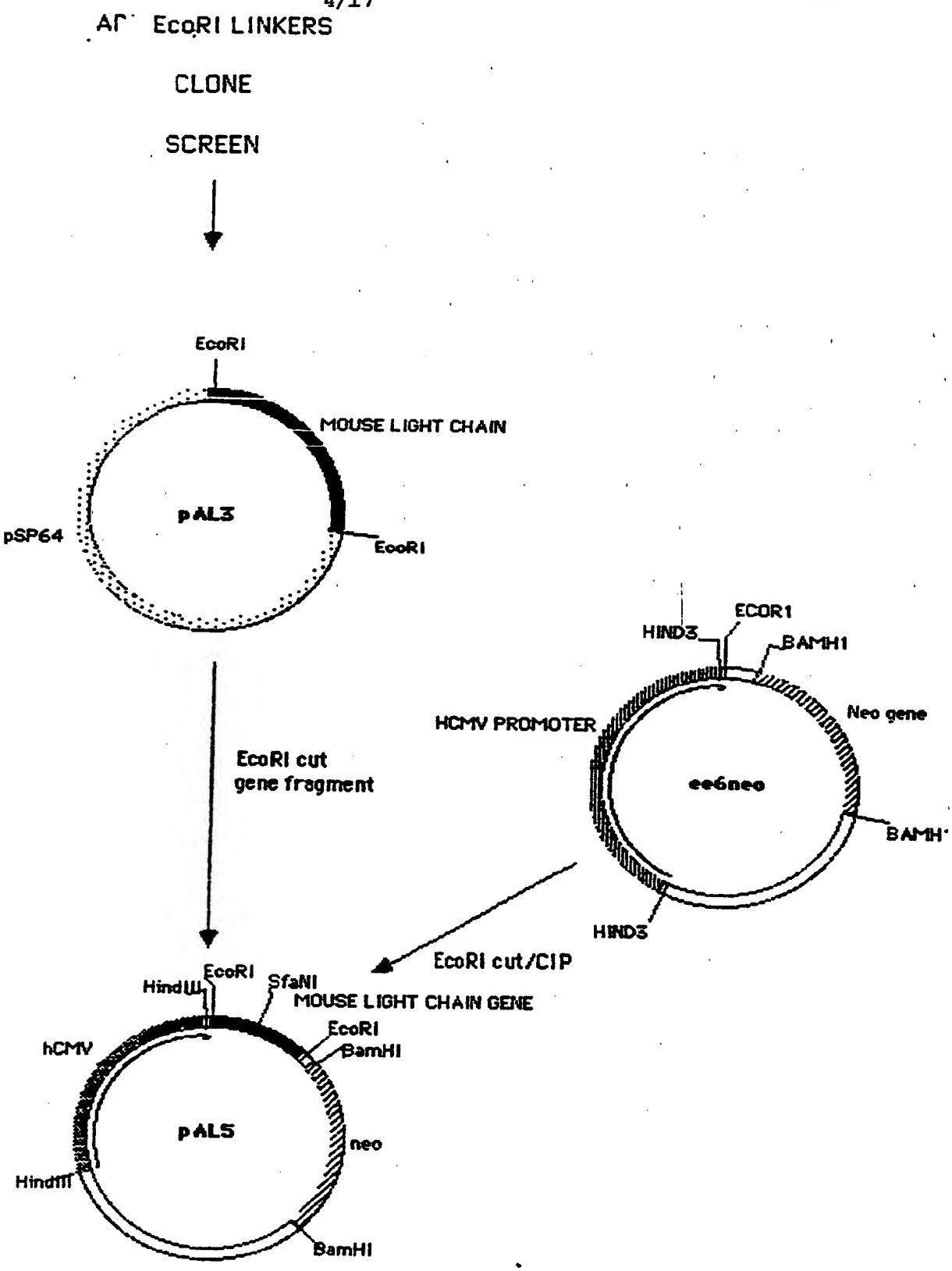


FIGURE 4
Figure 4 shows the process of formation of the anti-ICAM light chain expression vector pAL5

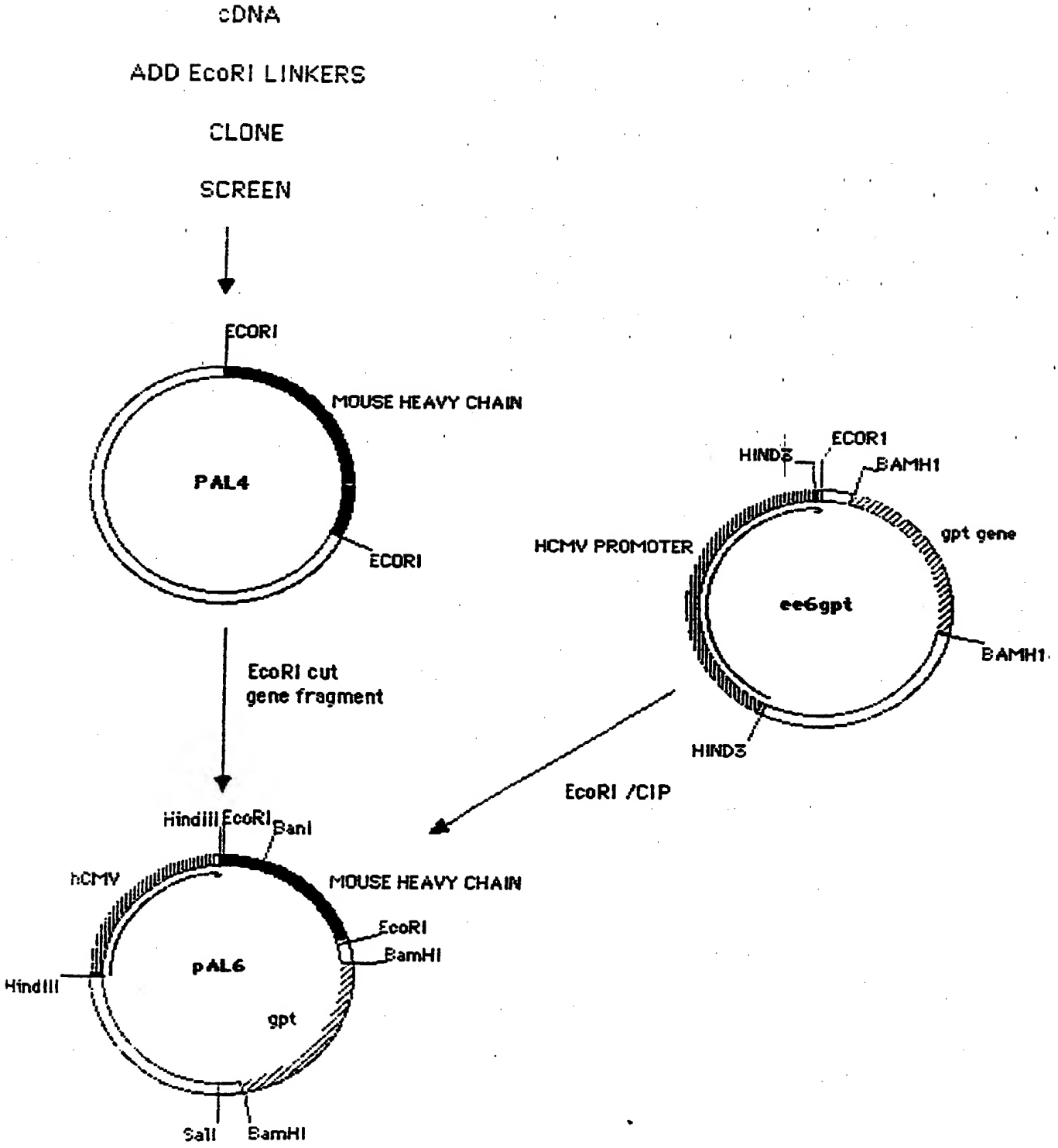
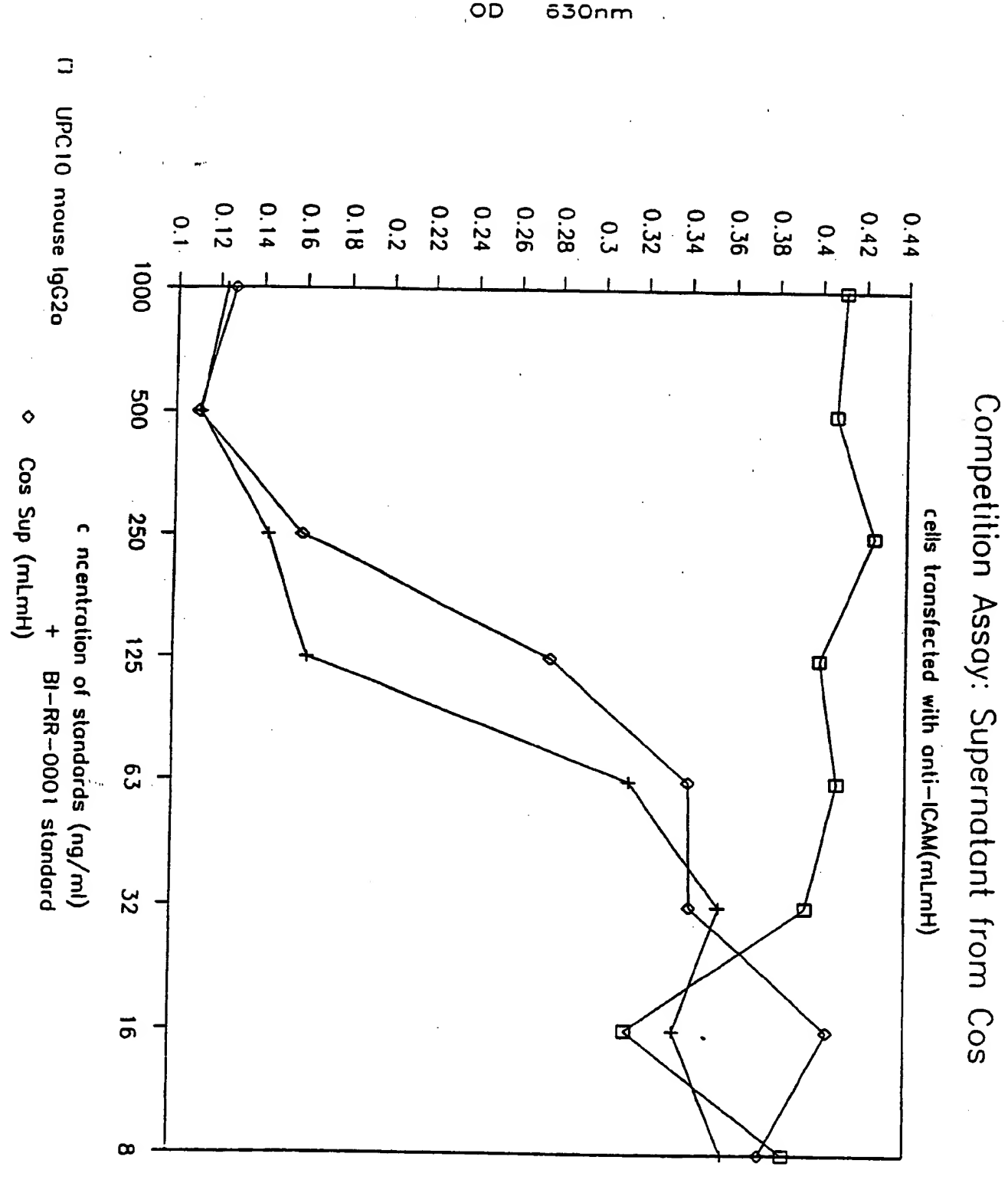


FIGURE 5
Figure 5 shows the process of formation of the anti-ICAM heavy chain gene expression vector

Figure 6



```
221     DIQMTQSPST LSASVGDRVT ITCRSSQSLV HSNGNNYLHW YQQKPGKAPK
221A    DVVMTQSPST LSASVGDRVT ITCRSSQSLV HSNGNNYLHW YQQKPGKAPK

221     LLMYKVSNRF SGVPSRFIGS GSGTEFTLTI SSLQPDDFAT YYCSQSTHVP
221A    LLIYKVSNRF SGVPDRFIGS GSGTDFTLTI SSLQPDDFGV YFCSQSTHVP

221     LTFGQGTKVE VKG
221A    LTFGQGTKVE VKG
```

Figure 7

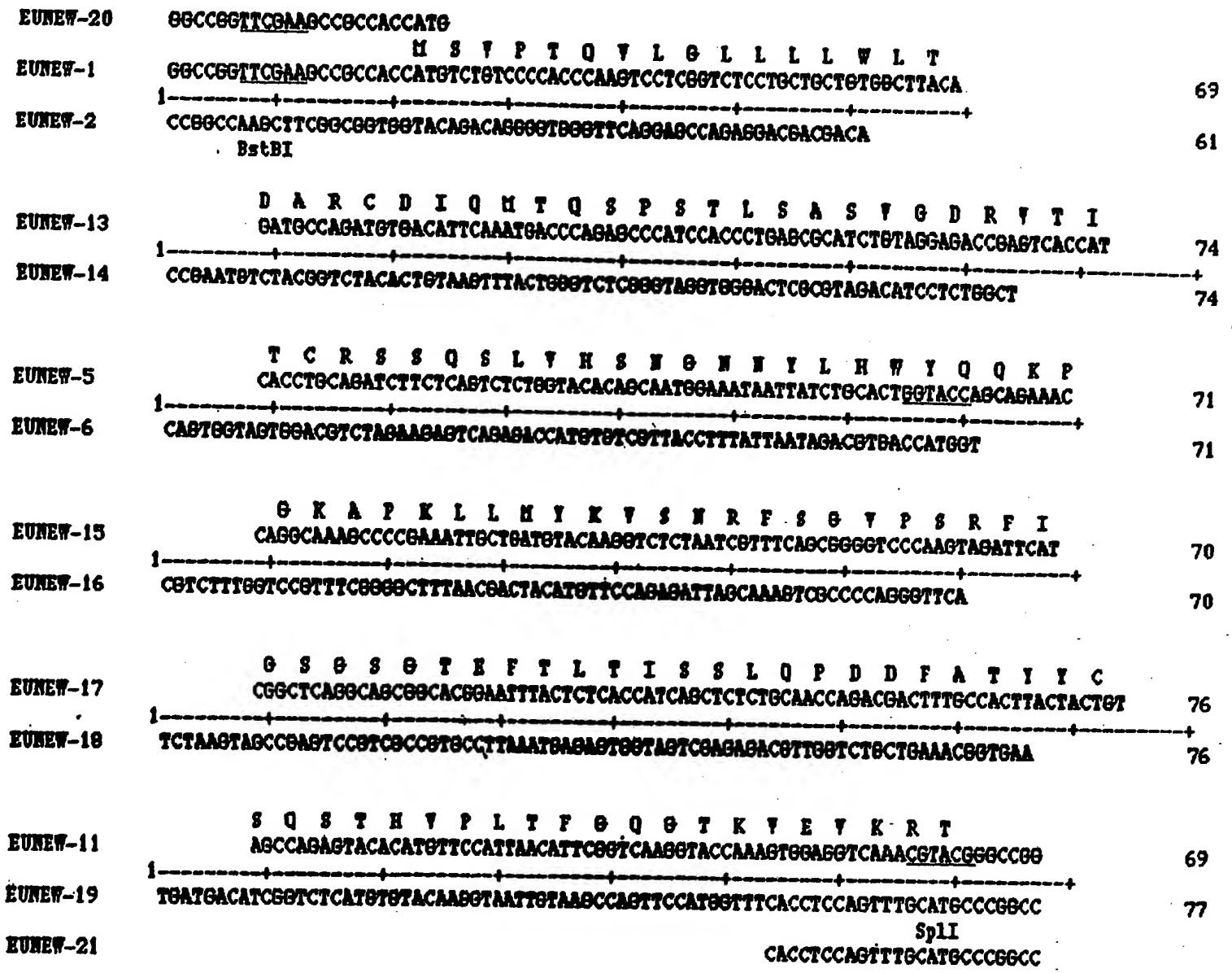

EUNEW-20
M S V P T Q V L G L L L L W L T
EUNEW-1
EUNEW-2
BstBI
D A R C D I Q M T Q S P S T L S A S V G D R V T I
EUNEW-13
EUNEW-14
T C R S S Q S L V H S N G N T Y L H W Y Q Q K P
EUNEW-5
EUNEW-6
G K A P K L L M Y K V S N R F S G V P S R F I
EUNEW-15
EUNEW-16
G S G S G T E F T L T I S S L Q P D D F A T Y Y C
EUNEW-17
EUNEW-18
S Q S T H V P L T F G Q G T K V E V K R T
EUNEW-11
EUNEW-19
SplI
EUNEW-21

Figure 8

Constructi n outline for anti-IC[illegible]
graft d light chain gL22

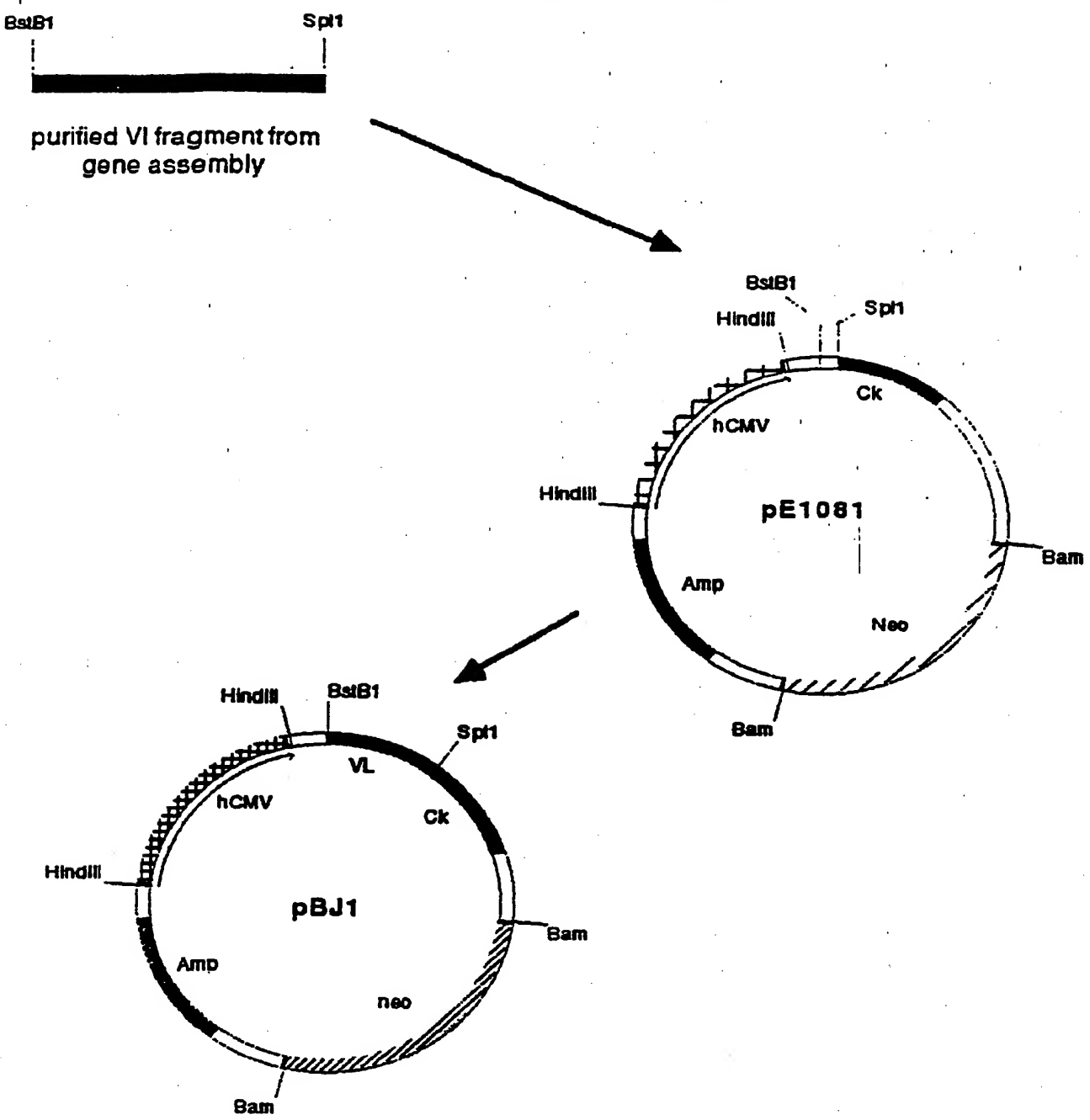


Figure 9

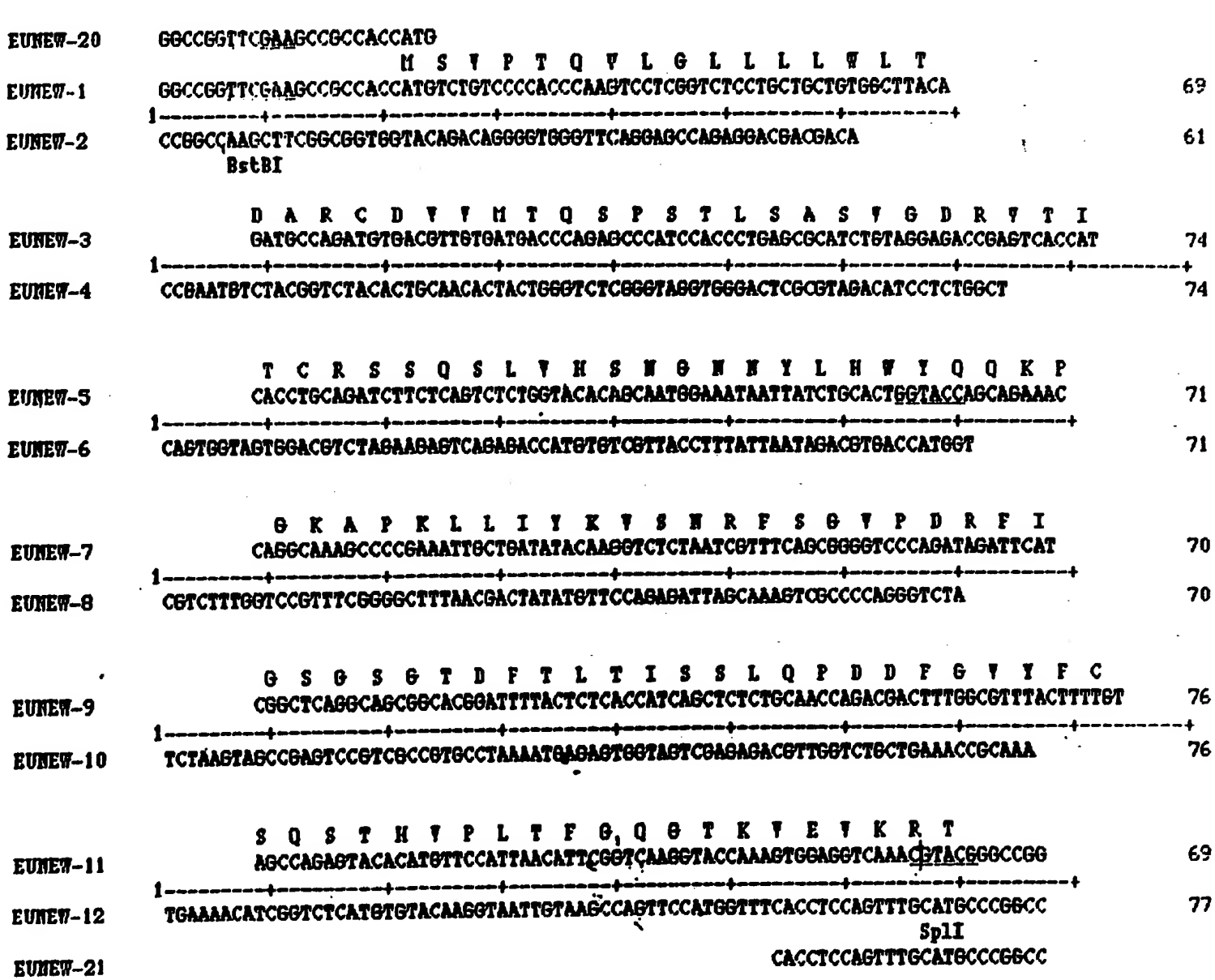


Figure 10

ICAM-EU HEAVY CHAIN ALIGNMENT.

```
                                        *cdr 1****                *
Icamh   QVQLQQSGPE LVRPGVSVKI SCKGSGYTFI DYAIHWVKES HAKSLEWIGV
gH341   QVQLVQSGAE VKKPGSSVKV SCKASGYTFI DYAIHWVRQA PGQGLEWMGV
gH341A  QVQLQQSGPE LVRPGVSVKI SCKGSGYTFI DYAIHWVRQA PGQGLEWMGV
gH341B  QVQLQQSGPE LVRPGVSVKI SCKGSGYTFI DYAIHWVRQA PGQGLEWIGV
gH341D  QVQLQQSGPE LVRPGVSVKI SCKGSGYTFI DYAIHWVRQA PGQGLEWIGV
Eu      QVQLVQSGAE VKKPGSSVKV SCKASGGTFS RSAIIWVRQA PGQGLEWMGG

        *****cdr2* ******                                    ***
Icamh   ISAYSGDTNY NQKFKGKATM TVDKSSNTAY LELARLTSED SAIYYCARGG
gH341   ISAYSGDTNY NQKFKGRVTI TADESTNTAY MELSSLRSED TAFYFCARGG
gH341A  ISAYSGDTNY NQKFKGRVTI TADKSTNTAY MELSSLRSED TAFYFCARGG
gH341B  ISAYSGDTNY NQKFKGRVTI TADKSTNTAY MELSSLRSED TAFYFCARGG
gH341D  ISAYSGDTNY NQKFKGRVTM TADKSTNTAY LELSSLRSED TAIYYCARGG
Eu      IVPMFGPPNY AQKFQGRVTI TADESTNTAY MELSSLRSED TAFYFCAGGY

        *cdr3***
Icamh   WLLLSFDYWG QGTTLTVSS
gH341   WLLLSFDYWG QGTTVTVSS
gH341A  WLLLSFDYWG QGTTVTVSS
gH341B  WLLLSFDYWG QGTTVTVSS
gH341D  WLLLSFDYWG QGTTVTVSS
Eu      GIYS..PEEY NGGLVTVSS
```

Figure 11

OLIGO'S FOR gH341 GENE CONSTRUCTION.

```
OLIGO NAME

                    M  E  W  S  W  V  F  L  F  F
B72EU1  AGCTTCCACCATGGAATGGAGCTGGGTCTTTCTCTTCTT                          39
     1  ---------+---------+---------+---------+---------+---------+
B72EU2        AGGTGGTACCTTACCTCGACCCAGAAAGAGAAGAAGGACAGTC               43

          L  S  V  T  T  G  V  H  S  Q  V  Q  L  V
B72EU3  CCTGTCAGTAACTACAGGAGTCCATTCTCAGGTGCAGCTCGTC                    33
     1  ---------+---------+---------+---------+---------+---------+
B72EU4            ATTGATGTCCTCAGGTAAGAGTCCACG                         33

                  Q  S  G  A  E  V  K  K  P  G  S  S  V  K  V  S  C  K  A
B72EU5            CAGTCTGGCGCTGAAGTGAAGAAGCCTGGGTCCTCAGTCAAGGTCTCCTGCAAGGCT 57
     1  ---------+---------+---------+---------+---------+---------+---------+
B72EU7  TCGAGCAGGTCAGACCGCGACTTCACTTCTTCGGACCCAGGAGTCAGTTCCAGAGGA       57

                  S  G  Y  T  F  I  D  Y  A  I  H  W                  CDR1
EU-NM1            TCTGGTTACACATTCATCGACTACGCTATTCATTGG                 36
     1  ---------+---------+---------+---------+---------+---------+---------+
EU2     CGTTCCGAAGACCAATGTGTAAGTAGCTGATGCGAT                           36

                  V  R  Q  A  P  G  Q  G  L  E  W  M
EU3               GTGAGGCAGGCTCCTGGACAGGGACTCGAGTGGATG                 36
     1  ---------+---------+---------+---------+---------+---------+---------+
EU4     AAGTAACCCACTCCGTCCGAGGACCTGTCCCTGAGCTCACCTACCCTCAATA           52
                                        XhoI
          G  V  I  S  A  Y  S  G  D  T  N  Y                        CDR2
EU-NM5  GGAGTTATATCAGCTTACAGCGGAGATACCAATTAC                          36
     1  ---------+---------+---------+---------+---------+---------+---------+
EU-NM6            TAGTCGAATGTCGCCTCTATGGTTAATGTTGGTCTT                 36

          N  Q  K  F  K  G  R  V  T  I  T  A  D
EU7     AACCAGAAGTTCAAGGGTAGAGTCACAATCACTGCAG                          37
     1  ---------+---------+---------+---------+---------+---------+---------+
EU8               CAAGTTCCCATCTCAGTGTTA                                21

                  E  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E  D
B72EU19           ACGAGTCCACGAACACAGCCTACATGGAACTCTCTTCTCTGAGGTCTGAGG  51
     1  ---------+---------+---------+---------+---------+---------+---------+
B72EU20GTGACGTCTGCTCAGGTGCTTGTGTCGGATGTACCTTGAGAGAAGAGACTC             51

                  T  A  F  Y  F  C  A  R
EU9               ACACTGCCTTCTATTTCTGTGCCAG                            25
     1  ---------+---------+---------+---------+---------+---------+---------+
EU10    CAGACTCCTGTGACGGAAGATAAAG                                      25

                  G  G  W  L  L  L  S  F  D  Y                        CDR3
EU-NM11           AGGAGGATGGCTGCTGCTGAGCTTCGATTAT                      31
     1  ---------+---------+---------+---------+---------+---------+---------+
EU-NM12ACACGGTCTCCTCCTACCGACGACGACTCGA                                 31

                  W  G  Q  G  T  T  V  T  V  S  S  A  S  T  K  G
J-TOP             TGGGGCCAGGGGACCACTGTCACTGTCAGCTCTGCTTCTACAAAGGGCC    48
     1  ---------+---------+---------+---------+---------+---------+---------+
J-BOT   AGCTAATAACCCCGGTCCCCTGGTGACAGTGACAGTCGAGACGAAGATGTTTC           52
```

Figure 12

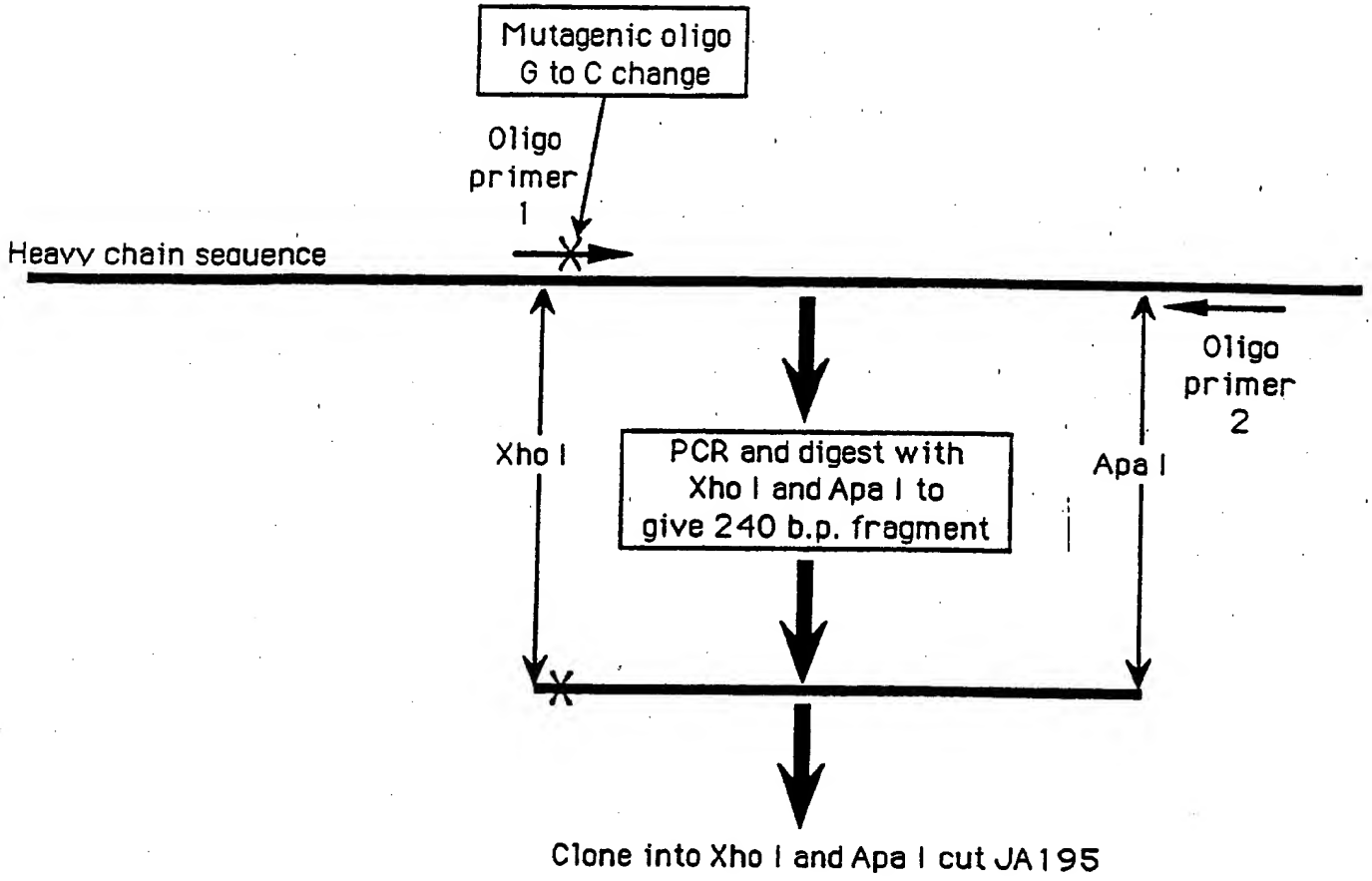


Figure 13

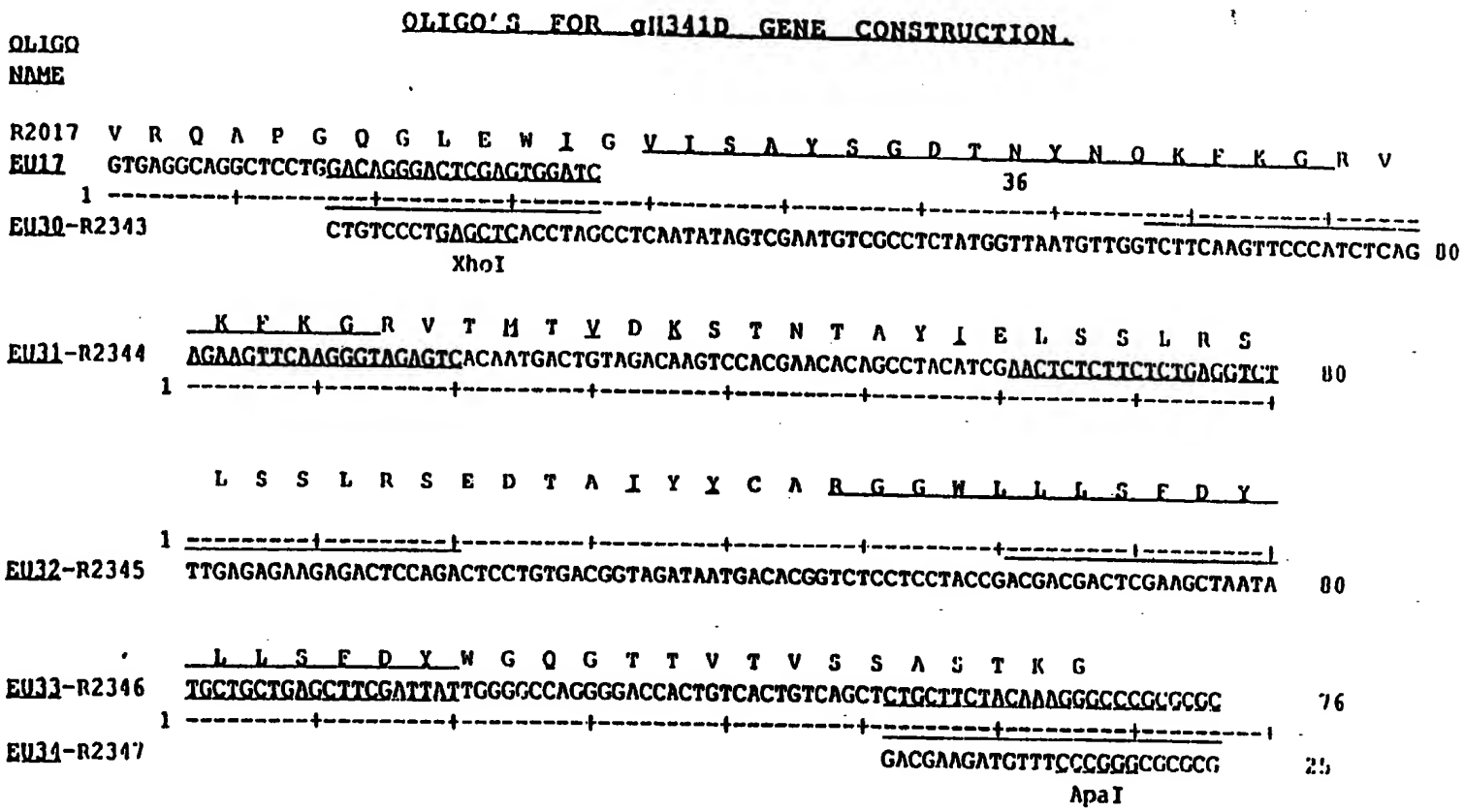


Figure 14

Figure 15

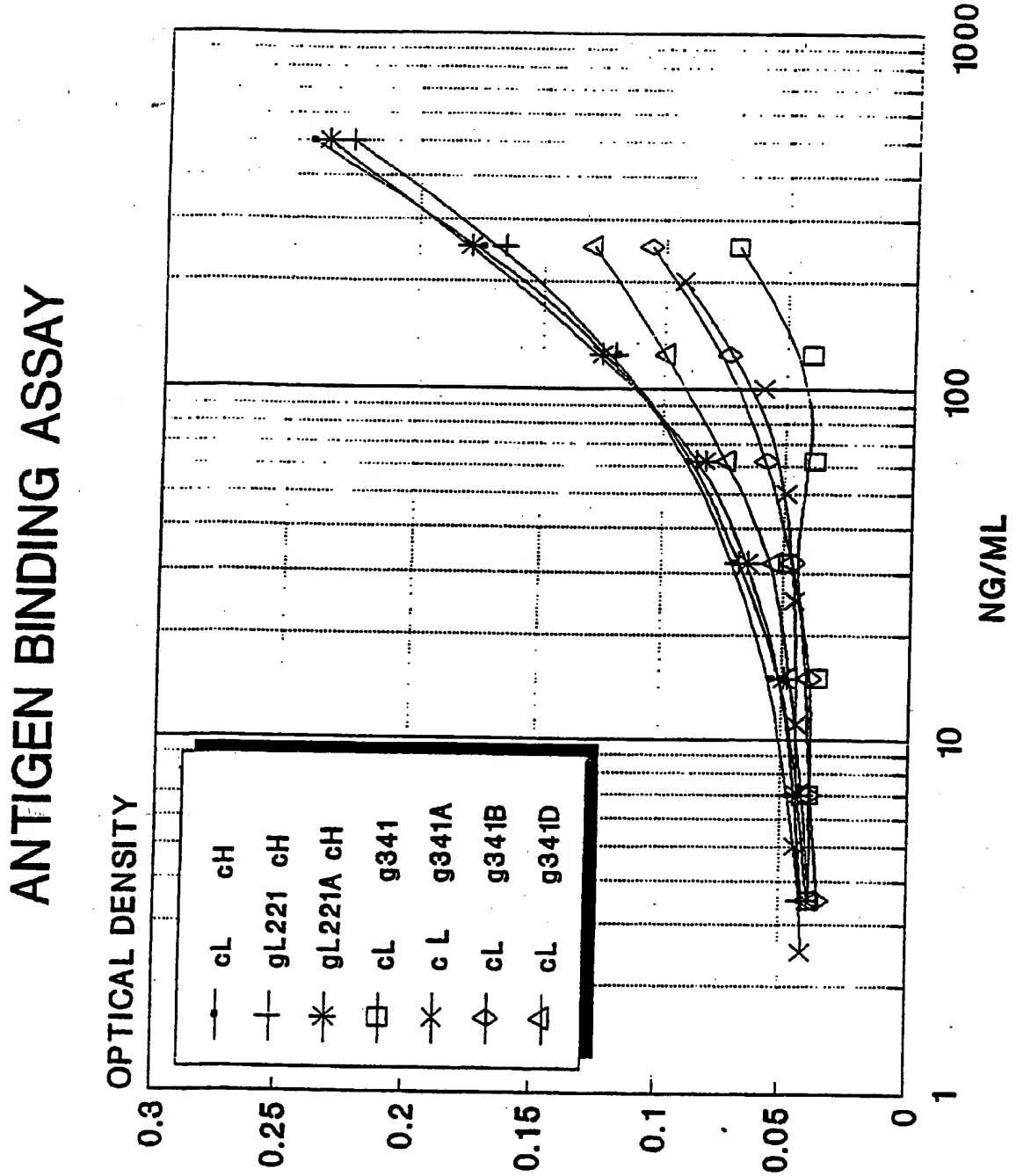


Figure 16

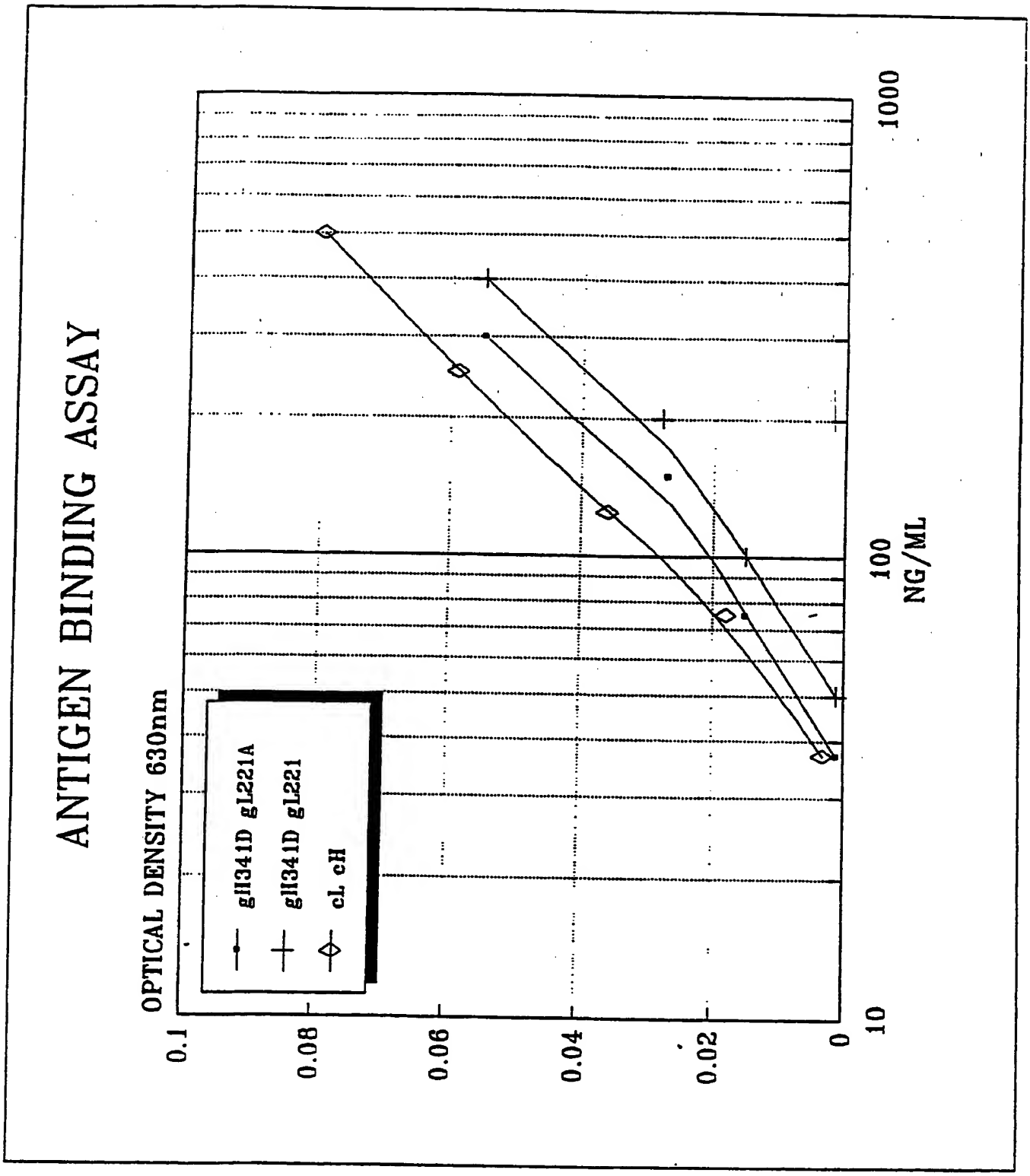
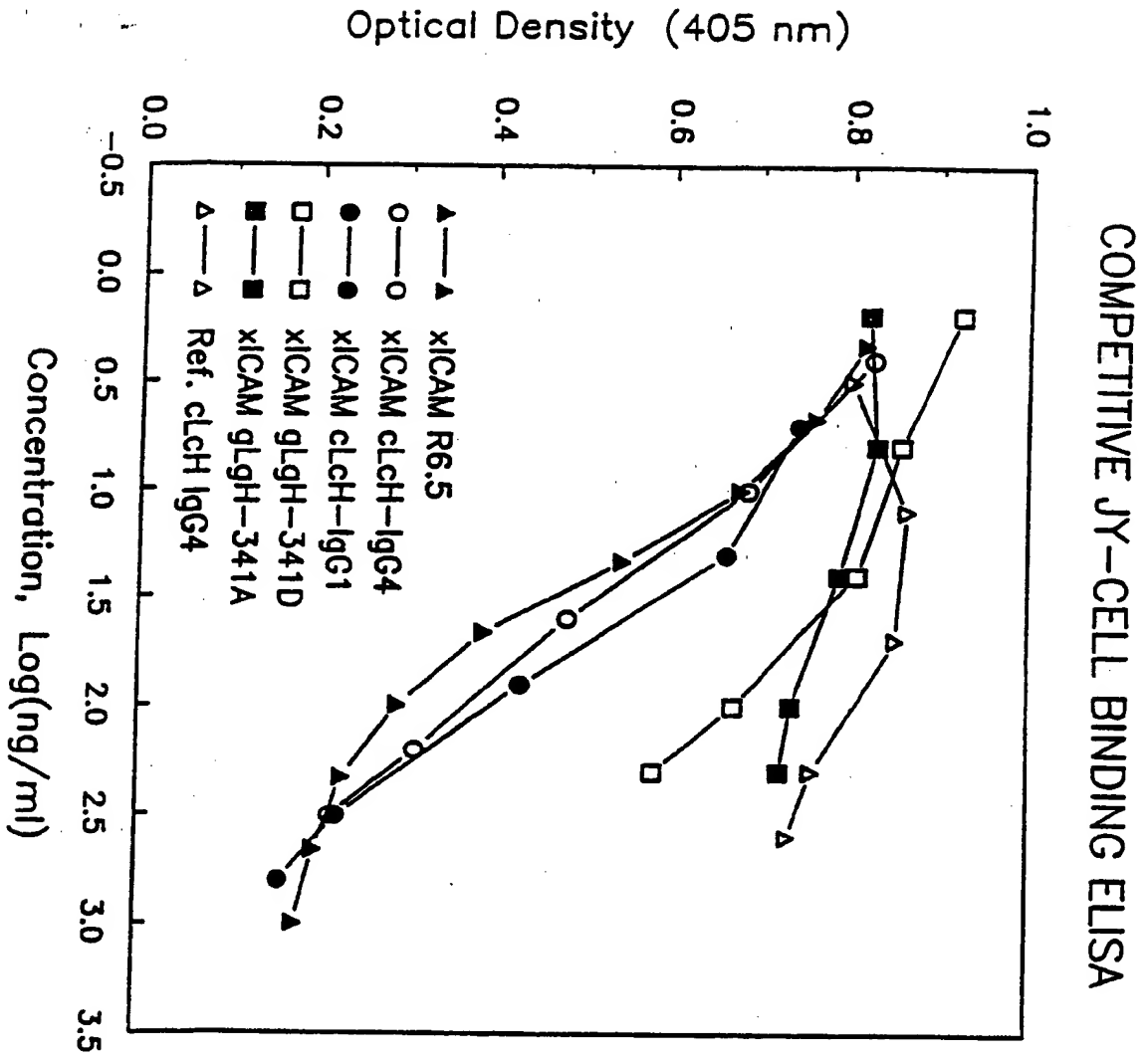


Figure 17



INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/02942

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 39/395; C07K 15/28
U.S. Cl.: 424/85.8; 530/387

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/387, 388, 389; 536/27 424/85.8, 88; 435/69.1,69.3,70.21,172.2,172.3,240.27,252.3,252.33,320.1;

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

Databases: Dialog (Files 5,73,155,351,357,399); USPTO Automated Patent Systems (File USPAT, 1971-1991).

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,816,567 (CABILLY ET AL) 28 March 1989, See entire document.	1-17,52-53
Y	JOURNAL OF CLINICAL INVESTIGATION, Vol. 82, issued November 1988, Smith et al., "Recognition of an Endothelial Determinant for CD18-dependent Human Neutrophil Adherence and Transendothelial Migration. pages 1746-1756. See entire document.	1-17, 19-32 48-53
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, Vol. 81, issued November 1984, Morrison et al., "Chimeric Human Antibody Molecules: Mouse Antigen-Binding Domains with Human Constant Region Domains, pages 6851-6855. See entire document.	1-17, 19-32 48-53
Y	NATURE, Vol. 321, issued 29 May 1986, Jones et al., "Replacing the Complementarily-determining regions in a Human Antibody With Those From a Mouse, pages 522-525. See entire document.	1-17, 19-32 48-53

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18 July 1991	20 AUG 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Robert D. Budens

Form PCT/ISA/210 (second sheet) (rev 11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, USA Vol. 86, issued December 1989, Queen et al. "A Humanized Antibody That Binds to the Interleukin-2 Receptor," pages 10029-10033, See entire document.	1-17, 52-53
Y	JOURNAL OF IMMUNOLOGY, Vol. 143, No. 4, issued 15 August 1989, Barton et al., "The Effect of Anti-Intercellular Adhesion Molecule-1 on Phorbil-Ester-Induced Rabbit Lung Inflammation," pages 1278-1282, See Abstract and Discussion.	19-32, 48-53
Y,P	EUROPEAN JOURNAL OF IMMUNOLOGY, Vol. 20, issued December 1990, Geissler et al., "A Monoclonal Antibody Directed Against the Human InterCellular Adhesion Molecule (ICAM-1) Modulates the Release of Tumor Necrosis Factor-α ,Interferon-γ and Interleukin 1," pages 2591-2596. See entire document.	19-32, 48-53
Y	WO, A, 89/01783 (CELLTECH) issued 09 March 1989, See Abstract	1-17, 52-53
Y	EUROPEAN JOURNAL OF IMMUNOLOGY, Vol. 20, issued February 1990, Buckle et al., "Human Memory T Cells Express Intercellular Adhesion Molecule-1 Which Can Be Increased by Interleukin-2 and Interferon-γ ," pages 337-341, See entire document.	1-17, 19-32 48-53

FURTHER INFORMATIC CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____. because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [4]

This International Searching Authority found multiple inventions in this international application as follows:

See attached sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

1-17,19-32,48-53 Telephone practice

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4 ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.